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HISTORY OF THE AMERICAN PHYSIOLOGICAL SOCIETY

To commemorate the Semicentennial of its foundation the American Physiological Society has published an illustrated history, with index, of its development since 1887. The book contains articles by William H. Howell and Charles W. Greene, with a foreword by Walter J. Meek, and was distributed in May, 1938.

The book of about 220 pages is in case-binding and costs $2.50.

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PHYSIOLOGICAL REVIEWS

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FUNCTIONAL ORGANIZATION OF THE SPINAL CORD

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The last decade has witnessed a major change in experimental approach to the problems of spinal cord function. Investigation has turned from myographic analysis of the reactions of peripheral effectors to the direct study, by electronic methods, of the spinal cord itself and its peripheral nerve paths. Attention has been directed to the fundamental properties of neurons and of synaptic regions, and also to the functional architecture of the spinal mechanism as it appears when played upon by various systems of reflex and higher control.

Recent developments in the study of nervous organization owe their measure of success to precise timing of events made possible by high speed, high fidelity recording methods, and to precise spatial localization of activity made possible by the development of refined microelectrode technique.

The central nervous system consists of primary afferent fibers, which transmit impulses from the periphery; motoneurons, which transmit impulses to the periphery; and interneurons connected in complex patterns with the afferent neurons and motoneurons. Potentially, activity entering the central nervous system by any given neuron path is capable of influencing by more or less devious routes every other neuron therein. In practice one finds a rather high degree of "local sign" attending reaction within the nervous system, a fact exemplified by the cortical response to tactile stimulation at a given peripheral locus (2), or the precise fractionation of the intrinsic spinal mechanism by various descending systems (36, 38). In addition to the spatial relations of a given response there are characteristic temporal relations between a stimulus and its action at a given point. An accurate accounting for the overall latent period for a given response is essential for an understanding of the pathway through which the response is mediated.

SYNAPTIC AND NUCLEAR DELAY. At first sight pools of neurons seemingly behave in a fundamentally different manner when activated by synchronous and asynchronous presynaptic activity. In the former case Lorente de Nó (47, 48) and others have shown that transmission occurs with a relatively fixed synaptic delay and total latency equals in time the sum of conduction time and synaptic delays. It is only in the simplest paths that such a reckoning represents the true state of affairs. A much longer delay is encountered at synaptic regions when activation results from a statistically smooth waxing and waning barrage of impulses. This longer time interval may be designated nuclear delay (36, 38). A clear understanding of the definitions and implications of the terms synaptic delay and nuclear delay is a useful preliminary for discussion of the problems of neural organization.

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The concept of synaptic delay introduced by Sherrington was quite adequate to, and in fact did, cover both types of delay recognized by contemporary description. When Lorente de Nó revealed the relatively fixed period of time lost by virtue of transmission through a single synaptic region under conditions of near-ideal synchronization, a new concept of synaptic delay was introduced. At the present time, and from a purely operational standpoint, synaptic delay is defined as the difference in time between transmission over a pathway of given length involving a synaptic relay, and transmission in a simple axon of equal length that has the properties of the parent presynaptic fiber for an equivalent length, and those of the postsynaptic fiber thereafter (cf. 57, p. 262). The minimum synaptic delay so defined in several alpha-tempo systems amounts to 0.5 to 1.0 msec. Such an interval of time is, however, a formal synaptic delay and includes time devoted to 'extrasynaptic' events, conspicuously conduction in the fine terminal branches and endings. From a theoretical point of view synaptic delay may be defined as the time interval between the arrival at the synapse of the last presynaptic impulse necessary to secure transmission and the onset of the resulting postsynaptic action. Once a full accounting is made for all the factors contributing to the so-called minimum synaptic delay it seems possible that synaptic time in the strictest sense will prove vanishingly small.

Frequently impulses from a given source may impinge upon a motor nucleus for several milliseconds before motor discharge results. Thus following stimulation of the brachial plexus excitatory impulses enter the motoneuron pools of the lumbar enlargement 2 to 3 msec. before the motoneurons discharge. During this interval the excitation of the motoneurons is subliminal, but progressively greater, as tested by direct (two-neuron-arc) reflex excitation, until discharge finally occurs. Since the term synaptic delay in current concepts is descriptive only of the relatively fixed time interval discussed above, it can no longer be used in the older sense to include the period of increasing excitation leading to discharge (cf. 15). For this period the term nuclear delay has been introduced (38). Nuclear delay may be defined as the time interval between the arrival at the synapse of the first presynaptic impulse to the onset of postsynaptic action. Under the condition of ideal synchronization of a given presynaptic volley it is clear that synaptic and nuclear delay would have identical values.

Since there is good reason to believe that successive impulses in the same presynaptic element are incapable of summation (46), nuclear delay is to be considered as a manifestation of the combined activity of a number of presynaptic units asynchronously active. Even so, by rotation of elements, repetition of presynaptic action may contribute to the rising level of excitability over a period of time.

The concept of nuclear delay is of the greatest importance in studies on functional organization since the arrival of the first impulses at a given point rather than the arrival of the last impulses defines the 'characteristic' pathway to that point from the site of origin of the transmitted activity. In short, central latency is equal to the sum of conduction time and nuclear delays (38).

THE LOCAL SPINAL MECHANISM. Although it is frequently regarded as such,

the local spinal mechanism is not strictly speaking a segmental structure, for the reflex collaterals from any given dorsal root ramify over a number of neighboring segments. Then too the interneurons supplied by a given dorsal root reach out to the white columns to embrace together a number of segments (7, 62). The reflex discharge through this mechanism, obtained by single shock stimulation of a dorsal root while recording from the ventral root of the same segment or a neighboring segment, displays a prominent initial peak, which is transmitted through arcs of two-neurons, i.e., directly from primary afferent fiber to motoneuron (13, 54). This peak is followed by an irregular discharge elevation of some 10 msec. duration, which is transmitted through multineuron-arc pathways. The two-neuron-arc reflex is more circumscribed than is the multineuron-arc reflex (41) which lends the additional weight of functional evidence to support the concept of circumscribed and diffuse reflex mechanisms proposed by Cajal.

It is impossible from ventral root recordings to know the peripheral distribution of the several components of the reflex discharge through the local spinal mechanism. So too, on stimulation of a dorsal root, fibers of many sensory modalities are activated together in unnatural combination. Therefore, the reflex discharges so obtained and recorded are of anatomical rather than of functional significance; they contain the elements of a number of ipsilateral reflexes, artificially admixed. One of the major problems in spinal organization has been the resolving of the local spinal reflex discharge into its functional components.

Associated with activation of the local spinal mechanism are 'slow' potentials which may be recorded by means of electrodes placed on or within the spinal cord, or by means of electrodes placed in close proximity to the cord on its associated dorsal and ventral roots. The analysis of these slow potentials provides one of the most difficult problems of neurophysiology and one which, on the basis of published work, has been accorded little critical attention. From the standpoint of the problems under discussion, experience has shown that the slow potential signs of activity are so widespread and so little understood as to be of little use in locating the active elements under any given conditions of excitation. Fortunately the use of refined microelectrode technique has never failed to reveal the presence of localized discrete spike potentials during the course of induced activity, even in the cerebral cortex where the existence of discharges of spike dimensions is rarely recognized.

THE CONSTITUTION OF AFFERENT NERVES. The dorsal roots contain fibers varying from approximately 20 micra to 1 micron in diameter (61). It has been found that only the largest of these, from 20 to 12 or 13μ, have direct connection with the motoneurons (39). The medium and small fibers of the dorsal roots contribute exclusively to the multineuron-arc reflex discharges. In the peripheral nerves major segregations of fibers occur, and these are of great reflex significance. The largest fibers are found only among the afferent fibers from muscle (61, 14, 52). In harmony with these histological observations is the fact that two-neuron-arc reflex discharges are provoked by stimulation of muscle afferent fibers and not by the stimulation of cutaneous nerves (39, 41). Medium size fibers (12–6μ) are found most prominently in cutaneous nerves (61, 14, 21, 53)

but are also present in muscle nerves. The finer myelinated fibers, those usually spoken of as delta fibers, again are to be found in both muscle and cutaneous nerves, being more prominent in the latter. Another group, consisting of unmyelinated fibers, may be included, for these are of proven reflex function (8, 4). The unmyelinated fibers form an impressive fraction of the total fiber count of a sensory nerve.

THE MYOTATIC REFLEX. Evidence has now accumulated to show that the reflex response to stretch is mediated through arcs of two neurons (39, 41, 42). One important characteristic of the myotatic reflex is that it appears only in the muscle or part of a muscle subjected to stretch (66, 33). Likewise it is a characteristic of the two-neuron-arc reflex that it appears only in the peripheral muscle nerve subjected to stimulation; it does not appear in other motor nerves nor in the nerve to one head of a muscle, the nerve to the other head of which is stimulated (41). Other experiments utilizing natural stimulation (42) have shown that the afferent response to stretch is transmitted in fibers of high conduction velocity (116 M. per sec.) and therefore of large diameter. Furthermore, it has been shown that the reflex response to sudden stretch is transmitted with but a single synaptic delay. The demonstration that a reflex evoked by a natural adequate stimulus is transmitted with no more central delay than that required for transmission of the segmental two-neuron-arc reflex resulting from dorsal root stimulation should dispose finally of any fears that the two-neuron-arc reflex is by nature an artefact, or that excitatory events other than the detonator action of impinging afferent impulses must antecede normal reflex transmission.

Two-neuron-arc reflex discharges are to be found also in association with flexor muscle pathways. The conditions for recording such discharges are precisely those obtaining in the case of extensor muscle pathways (41). The reflex represented by such activity is best known as the 'pluck' reflex or flexor tendon-jerk (1).

THE FLEXOR REFLEX. While the reflex effect attending stimulation of the low threshold muscle afferent fibers is confined to two-neuron-arc pathways, the reflex discharge following stimulation of the medium and small myelinated fibers of either cutaneous or muscle nerves has all the attributes of the multi-neuron-arc discharge as encountered in the segmental reflex. In distribution the resulting reflex discharge is largely restricted to the motor nerves of flexor muscles, and represents the flexor reflex. The shortest pathway for transmission of this reflex discharge involves an intercalated neuron between the primary afferent fibers and the motoneurons (41). Until recently it has been supposed that direct connections between afferent fibers and motoneurons serve flexor reflexes as well as stretch reflexes. In consequence the functional significance of the circumscribed and diffuse reflex mechanisms as conceived by Cajal from histological evidence has been overlooked. It is now clear that the direct and restricted two-neuron-arc pathway (the circumscribed reflex of Cajal) serves to limit to the muscle stretched the excitatory reflex field of action of the afferent influx from tension receptors. In contrast, the interneuron intercalated in the minimum flexor reflex pathways provides the structural mechanism of insuring the diffusion of the flexor reflex to many or all the synergically acting muscles (the diffuse reflex of Cajal).

One of the most interesting problems awaiting solution is that of relating the sensory modalities and reflex effect. It appears that even the largest fibers in cutaneous nerves, when stimulated, contribute to the flexor reflex, as do the smaller fibers of the delta group. The flexor reflex is customarily regarded as the response to painful stimulation, although final justification for a specific relationship is not yet available. There seems to be general agreement that delta fibers carry pain producing impulses (cf. 19), but Zotterman's experiments with touch stimulation (75) indicate that delta fibers carry touch as well as pain. If all the delta fibers contribute to the delta flexor reflex, it follows that touch as well as pain can promote flexion of the limb, but there is no certain evidence that this is the case.

Again, in summarizing the experimental evidence relative to pain fibers, Gasser (19) finds indications that there must be some fairly large-sized pain fibers. Such fibers may account for the flexor reflex obtained by weak stimulation of cutaneous nerves, but since the range of fibers above delta accommodates temperature and pressure fibers, it is impossible to say what modalities are represented in a stimulated volley relayed from afferent fibers into the flexor reflex connections. A partial solution to these problems might be forthcoming if it were known to what extent the medium and small myelinated afferent fibers, by collaterals, feed into the local reflex mechanism.

Similar problems attend the correlation of sensory modalities represented by the medium and small afferent fibers arising in muscle with the reflex effect of stimulating these fibers (flexion). Presumably some of these fibers in certain situations are concerned with the mediation of the lengthening reaction.

THE EXTENSOR THRUST AND REFLEXES OF CROSSED EXTENSION. At the present time there is little systematic knowledge of the central mechanism of these two reflexes. It is known from the work of Sherrington (62, 63, 64, 66), that the afferent pathway for the extensor thrust reflex is contained in the plantar nerves, but stimulation of these nerves evokes only flexion (66) and discharges into flexor nerves. There is good reason for stating that the extensor thrust (41) and the crossed extensor reflex (50) are both transmitted through multi-neuron-arc pathways, but certain knowledge of the minimum pathway and relay points is not as yet available. In the case of the extensor thrust it would seem that this knowledge will only be obtained by the use of natural stimulation.

ACTIVITY IN LONG SPINAL REFLEX PATHWAYS. Reflex action from forelimb to hindlimb is mediated conspicuously by the long spinal fibers described by Sherrington and Laslett (67). In chronic spinal animals the intrinsic mechanisms of the spinal cord may be studied in isolation from the extrinsic mechanisms by virtue of degeneration of the latter. Direct stimulation of the ventrolateral tracts in such preparations shows that the long spinal fibers of the ventrolateral columns conduct impulses at alpha velocity, and that these impulses are relayed into the short spinal fibers of the ventrolateral columns (43). The relationship of long spinal fibers and short spinal fibers, then, is similar to the relationship between supraspinal (bulbospinal) fibers and short spinal fibers (34, cf. below). Additional information as to the activity of the spinal system has been gained by reflex activation of the system rather than by direct stimulation of the tracts

themselves (38). When activated reflexly, the discharges descending through the spinal system possess the diffuse waxing and waning nature of nuclear discharge in contrast to the synchronized volley resulting from direct stimulation.

Aboral transmission of the activity evoked by reflex stimulation on one side of the body involves intrinsic spinal tracts of both halves of the spinal cord, indicating a free decussation within the brachial field of the cord. Activity eventually reaches motoneurons of the lumbar region by strictly ipsilateral paths, by crossed paths, and by the recrossing in the lumbar region of crossed activity, due to the free yoking of the halves of the cord in the lumbar enlargement. The lateral columns are concerned with strictly unilateral transmission. The ventral columns subserve bilateral as well as unilateral transmission. The dorsal columns appear not to be of primary importance.

Internuncial activity, resulting in the lumbar enlargement from afferent volleys to the cervical enlargement, is confined to the ventral horn. Conspicuous among the active neurons are those constituting the nucleus of the anterior commissure, which fact is in harmony with the observed degree of importance to be attached to crossed conduction of long spinal reflex activity.

Some motoneurons of the lumbar enlargement are inhibited at the calculated time of arrival of the first long spinal impulses at the site of action. With the onset of internuncial activity in the ventral horn of the lumbar enlargement certain two-neuron-arc reflexes are facilitated and, after a few milliseconds nuclear delay, motoneurons begin to discharge in response to the long spinal reflex excitation.

The Schiff-Sherrington phenomenon provides an interesting and somewhat perplexing instance of long spinal action in the ascending sense (59).

ASCENDING PROJECTION SYSTEMS. Many papers are to be found dealing with the cortical response, cerebral and cerebellar, to stimulation of peripheral nerves, or to natural stimulation, superficial or deep, but only in a very few cases has any attention been accorded the spinal mechanism mediating the cortical response.

The dorsal columns. The mean maximal conduction rate of impulses ascending through the dorsal columns approximates 70 M. per sec. (25). Since many afferent fibers conduct impulses at rates approaching 120 M. per sec. (42) either a selection of neurons or a change in conduction properties occurs soon after the dorsal root fibers reach the posterior columns. Gasser and Graham (20) described the slowing of velocity taking place within a few centimeters of the root entry, and attributed it to decrease in diameter of the axons after giving off collaterals to the gray substance. While this is undoubtedly the case, it is well to bear in mind the possibility that the dorsal columns contain significant selections from among the fibers of the dorsal roots, the largest fibers perhaps extending only far enough to plunge in to the Clarke-Stilling nuclei.

The long tracts of the dorsal columns terminate in the nucleus gracilis and nucleus cuneatus. The outstanding feature of transmission through the nucleus cuneatus is the powerful 'one-to-one' relay to the medial lemniscus (70). Activity of more complex origin is also present in the medial lemniscus, part of which may be ascribed to the ascending homologue of the dorsal root reflex described by Hursh (30). All except the initial relay is easily inhibited by repetitive activa-

tion, with the end result that transmission of a tetanic series through the nucleus cuneatus takes the form of a series of synchronized 'jets' into the medial lemniscus.

The dorsal spino-cerebellar (Flechsig's) system. According to the analysis of Grundfest and Campbell (25) the origin of Flechsig's tract in the column of Clarke receives further confirmation. The tract fibers reach out from the column of Clarke to the dorso-lateral surface of the lateral column and ascend to the medulla as a flat ribbon of fibers. For further detail of the fine structure of this tract the important paper of Sherrington and Laslett (68) should be consulted. Grundfest and Campbell (25) find that Flechsig's tract is activated by stimulation of muscle afferent fibers, but not by stimulation of skin afferent fibers. This fact would suggest that the large afferent fibers which are responsible for transmission of the stretch reflex (41, 42) and the direct inhibitory actions of dorsal root fibers (39) also supply the cells of Clarke's column. There seems to be no reason to suppose that distinct afferent fibers supply the stretch reflex and the proprioceptive projection to the cerebellum, and in all probability by collateral connection Flechsig's tract is activated *pari passu* with stretch reflexes.

It is important to recall the observation of Schäfer (60) that lesions involving the pyramidal tract (and presumably other descending fibers) result in the appearance of large numbers of degenerated fibers "passing from the pyramidal tract (below the lesion) towards Clarke's column, curving round the ventral aspect of the section of that column and losing themselves as fine (probably branching) fibres amongst its cells." In general, Schäfer's viewpoint is so strikingly confirmed by recent functional studies on the pyramidal tract (36), that one must consider the interesting possibility that higher (i.e., descending) systems which influence the tendon-jerks may also influence the ascending concomitant of the tendon-jerk at its point of relay in Clarke's column. The possibility would appear to merit careful investigation.

Impulses in Flechsig's tract are conducted at high velocities by large fibers (cf. 25, 26), with the result that they soon outstrip dorsal column impulses evoked in parallel, but not similarly subjected to synaptic relay with consequent loss of time (25). The earliest components of the cerebellar response to stimulation of a mixed nerve (tibial) are transmitted through Flechsig's tract, for section of it blocks them. The late component of the cerebellar response, in contrast, is not so blocked, nor is the (late) response provoked by stimulation of the purely cutaneous saphenous nerve. Presumably the late component resulting from stimulation of the tibial nerve is the result of stimulation of the cutaneous afferent fibers in that nerve. In this connection it is of interest that a number of workers (11, 25, 12, 69) have recently reported cerebellar responses to stimulation of skin nerves or to natural tactile stimulation. Grundfest and Campbell's results show that the cerebellar response to stimulation of cutaneous afferent fibers is mediated by some other than Flechsig's tract.

Other ascending systems. Little is known, or may be conjectured as to the functional aspects of other ascending systems of the spinal cord. Many such systems consist of fine fibers, which places difficulties in the path of investigation.

In a recent paper, Cooper and Sherrington (9) describe chromatolysis of large

cells scattered along the margin of the ventral horn following contralateral hemisection in the cervical or thoracic cord. Tower, Bodian and Howe (73) likewise found chromatolysis of the 'spinal border cells' after isolation of a segment of the spinal cord. According to the evidence of Cooper and Sherrington the spinal border cells give rise to an ascending decussating tract which they interpret to be Gower's tract (ventral or indirect spino-cerebellar tract). Since Cooper and Sherrington find some chromatolysis of the crossed Clarke's column it is still possible that this column contributes a part of the inflow to the tract of Gower.

DESCENDING PROJECTION SYSTEMS. *The ventrolateral descending tracts.* The projection through the dorsal longitudinal bundle to the ventrolateral columns of the cord derives largely from the vestibular and reticular nuclei. The major influences mediated through this system, conveniently termed the bulbospinal correlation system, are vestibular (and cerebellar) in origin.

The tract fibers of the bulbospinal correlation system are rather uniform in size and possess a high conduction velocity, as judged by the absence of dispersion in a volley of impulses conducted from the dorsal longitudinal bundle to the sacral cord, a distance of some 30 centimeters (34). The bulbospinal fibers when activated in isolation have relatively little direct action upon the motoneurons, but do exert a powerful driving force upon the large fiber short propriospinal or intrinsic spinal neurons of the ventral horn. Impulses from the long tracts are relayed, apparently at all levels into the ventral horn interneurons (short spinal neurons) which in turn play powerfully upon the motoneurons. Backing up this powerful three-neuron pathway from the medulla to the periphery is a wealth of asynchronous internuncial discharge, probably confined to the ventral horn, which by "reverberating" onto the short spinal nuclei, provides for the observed powerful facilitation of those nuclei with the advent of repetitive stimulation.

Motoneuron discharge resulting from the convergence of tract volleys initiated in the bulbospinal system and local internuncial activity resulting from similar volleys is controlled in time by the action of the short spinal neurons. On the other hand, when the same tract volleys converge with internuncial activity derived from primary afferent volleys, all other conditions being equal, motoneuron discharge advances from the control of the short spinal neurons to that of the primary tract fibers, and the direct functional pathway from the tract fibers to the motoneurons supersedes the indirect pathway through the short spinal neurons. The available evidence unfortunately does not permit one to say whether the motoneurons controlled by the direct tract action and those controlled through the short spinal fibers supply muscle of the same or of opposed action. The significance of the experimental finding accordingly is obscure. If the motoneurons discharged in each case are of different, and perhaps reciprocal, function, then it seems probable that the actions just described provide an essential mechanism for the production of rhythmic alternating activity continuously under the control of a single projection system.

The pyramidal system. The pyramidal tract consists of those fibers descending through the medullary pyramid to the spinal cord (cf. 74, 17, 48). It is, com-

pared with other tracts that have been studied, a pathway of slow conduction velocity, at any rate in the cat. The upper limit of the range of conduction velocities lies between 60 and 65 M. per sec. Velocities as low as 18 M. per sec. may be assigned to pyramidal tract fibers with reasonable certainty. Assuming that the diameter-velocity relations described for peripheral nerve fibers (21, 29) hold for fibers in the central nervous system, it is likely on the basis of histological evidence that fibers of still slower conduction velocity are represented, but their presence has not been demonstrated unequivocally by means of recorded action potentials.

On stimulation of the medullary pyramid, after suitable precautions to prevent impulses in any tracts other than pyramidal from entering the spinal cord, it is found that interneurons at the base of the dorsal horn immediately adjacent to the pyramidal tract become active (cf. the conclusion of Schäfer based on the evidence of degenerations (60)). The discharge of this nucleus by single shock stimulation of the pyramidal tract is insufficient to provide further spread of the activity, for no other activity is found on careful exploration with a microelectrode, nor are local reflexes in any way influenced. With repetitive stimulation activity is intensified and spread to other nuclei occurs. When the system is fully active internuncial responses are confined to the dorsal horn and intermediate region. The arrival of excitatory impulses at the intermediate region is accompanied by facilitation of three-neuron-arc (i.e., flexor) reflexes, without any parallel effect upon motoneurons observable. It is only after a nuclear delay of several milliseconds that discharges within the intermediate region occur, and facilitation of motoneurons parallels this discharge in the intermediate region. From this evidence it would appear that the primary action of the pyramidal fibers, active in isolation, is on the flexor reflex arcs.

Corticospinal effects. Activity transmitted into the spinal cord directly through the pyramidal tract forms only a part of the total effect in the spinal cord of stimulating the pericruciate area of the (cat's) cerebral cortex. Tower (72) for instance, has described actions, both motor and inhibitory, on stimulating the pericruciate area (and other areas) after section of the medullary pyramids, and suggests the possibility that the reticulospinal tracts are involved in mediation of these extrapyramidal actions.

One of the most striking sequelae of single shock stimulation of the motor cortex of the cat is the widespread activation of the reticular formation after a latency of approximately 4 msec. (43). The exact pathway from cortex to reticular formation is not yet known but on the basis of time relations it is quite direct. The reticular activity is in turn projected by high velocity tract fibers to the ventral horn of the spinal cord. It follows, then, that cortical control of the dorsal and ventral halves of the local spinal mechanism is dissociated at a prespinal level, impulses to the dorsal half being directly conducted by the pyramidal fibers themselves, those to the ventral half being relayed by reticular elements. A simple calculation based on the known conduction rates and delays involved shows that cortically evoked activity through the reticular relays would reach the local spinal mechanism coincidentally with and earlier than the directly

conducted pyramidal impulses proper. There is, then, the further possibility that cortical impulses and vestibulo-cerebellar impulses converge at the prespinal level, and then by the high velocity projection system 'set the stage' for the play of the pyramidal impulses arriving through the slower conducting pyramidal fibers.

ALPHA FIBER SYSTEMS AND POSTURAL CONTROL. Considerations such as that above serve to emphasize a correlation which seems of great potential significance (18, 19). The myotatic reflex pathway, and its associated projection through Flechsig's tract to the cerebellum, the vestibular and reticular systems and the dominant descending long spinal reflex pathways all have several dynamic properties in common. They are characterized by the large size, high conduction velocity and relative uniformity of their constituent nerve fibers, and they are all primarily concerned with the transmission of postural activity. In contrast, other reflex, afferent and motor systems, those mediating flexor reflexes, cutaneous sensitivity and voluntary motor activity are characterized by diversity of fiber size, with the largest fibers not overlapping to any great extent the range present in the postural mechanisms. In general too the synaptic connections of these systems are more devious and complex than those of the postural mechanisms. One is tempted to speculate on the functional significance of these facts for the integration of movement in the intact animal. It seems quite plausible that the function of the high velocity systems set in parallel with other paths is in part to antecede those paths in temporal sequence of action and so to adjust posture to a state appropriate to the initiation of movement in readiness for the actual performance of that movement.

INHIBITION. Perhaps the thorniest of all problems connected with the study of nervous organization is that of clarifying the mechanisms of inhibition. In general theories of inhibition postulate either a block of some part of the reflex pathway as the sequel to discharge or an inhibitory action of a direct nature for which the prototype is the long known vagal effect on the heart. A theory (22) but recently expanded by Gesell (23) holds that there is a constant flow of current between dendrites and axon hillock, and that the action of impinging impulses (all presumably alike) is to increase or decrease the flow of current depending upon the anatomical relationship between the active synaptic knobs and the several parts of the responding neuron. Depending upon the change in the current flow, either increasing or decreasing, the neuron is excited or inhibited. Barron and Matthews (3) have suggested that polarization of axon junctions by adjacent activity may prove to be a true mechanism of central inhibition.

Hughes and Gasser (28) and Gasser (18) point out that the subnormal period, discovered first in nerve by Graham (24), would account for the inhibition of the flexor reflex to the second of two successive single shock stimuli (16). In the same vein, Lorente de Nó (45) showed that a group of motoneurons, after antidromic activation, exhibit a prolonged period of subnormality, an event sufficient to account for the silent period following the transmission of a tendon-jerk reflex. An important consideration for the occurrence of inhibition by the intervention of the subnormal period lies in the necessity for other than syn-

chronous convergence of interacting volleys, which event *ceteris paribus* would lead to summation.

Following the demonstration by Hughes and Gasser (28) that the negative cord potential, signalling the activity of interneurons, and the reflex discharge to the second of two successive volleys are decreased in a parallel manner along a time course which follows that of the positive cord potential resulting from the first volley of the pair, McCouch, Hughes and Stewart (51) discovered that crossed inhibition of the flexor reflex may occur with little or no inhibition of the cord potential as recorded from the dorsal surface of the spinal cord, which fact leads them to the conclusion that inhibition in that case occurred at the motoneurons without conditioning by previous activity of the units involved. Although this indeed may be the case, there is no evidence as yet by which to exclude other than direct inhibitory actions. To interpret the experiments of McCouch, Hughes and Stewart it would seem important to know whether all of the interneurons which are required, in one way or another, for the transmission of the flexor reflex contribute sufficiently to the cord potential that their response may be assessed by inspection of the cord potential. It does seem likely that the experiments of McCouch, Hughes and Stewart preclude the possibility of crossed inhibition of the dorsal horn, whatever the mechanism, as the essential event in crossed inhibition of the flexor reflex, at least under the conditions in which the cord potential is not reduced with the reflex.

Although it is valid to associate the negative intermediary potential and the flexor reflex, for the afferent components giving rise to the two events are similar (41), whether the association is one of casual relationships or of parallel relationships is not clearly defined by experiment. Peripheral stimulation which gives rise to reflex flexion also gives rise to sensation, usually but not of necessity said to be painful. There is seemingly no evidence upon which to state unequivocally whether the internuncial activity represented by the negative intermediary potential is primarily concerned with mediation of the flexor reflex or with mediation of sensation provoking impulses to ascending projection systems.

Several examples of direct inhibition recently have been discovered, in each case the condition for the onset of inhibition being simultaneous convergence of the interacting volleys. As first described, a single afferent volley in the sixth lumbar dorsal root was found to inhibit the two-neuron-arc reflex evoked in the first sacral ventral root by a single volley in the first sacral dorsal root when the two dorsal root volleys were synchronized (35). Other examples of this form of central inhibition have since been disclosed (56, 38), and with one exception the inhibitory action has been unilateral and ipsilateral. The exception was found in experiments on the third sacral segment in which a dorsal root volley on one side of the cord will inhibit the two-neuron-arc reflex evoked by a dorsal root volley on the other side of the cord when the two dorsal root volleys are synchronous (43). This crossed inhibition appears to be related to the bilateral nature of the effectors innervated by this segment.

The afferent fibers yielding direct inhibition to the motoneurons are indistinguishable from those responsible for the two-neuron-arc reflex discharge (39),

and belong, therefore, to the group of large low-threshold afferent fibers arising in muscle. Although a systematic survey has not yet been made, it appears from the known examples of direct inhibition that the low-threshold afferent fibers from a given muscle mediate two-neuron-arc excitation to the motoneurons of that muscle and direct inhibition to those of neighboring muscles. It is apparent, then, that the direct inhibitory action of muscle afferent fibers accounts for the silent period shown by Denny-Brown to occur in the gastrocnemius muscle as the reflex accompaniment of the tendon jerk in the quadriceps muscle (10).

The fact that inhibition of two-neuron-arc pathways occurs when stimulating and testing volleys are synchronous is in itself strong evidence for the existence of some form of direct inhibitory action, but still further evidence may be found in the fact that this inhibition occurs with threshold conditioning volleys (39), which are well below the size requisite to produce motoneuron discharges. If near-threshold conditioning dorsal root volleys are utilized the period of depression of a two-neuron-arc test reflex volley may last for 7 or 8 msec. (43) whereas, with larger conditioning volleys internuncial pools are powerfully activated and inhibition is truncated by the advent of facilitation. When a more physiological selection of motoneurons is made by utilizing an extensor muscle nerve rather than ventral roots for recording, an inhibitory period of more than 100 msec. may be realized (56) which suggests the participation of interneurons in the inhibitory conditionary activity.

In spite of the proven fact of direct inhibition, nothing that is yet known serves to indicate the mechanism entailed. There is no reason to suppose that distinct inhibitory fibers exist in the mammalian spinal cord, for the threshold and fiber spectrum of direct excitation and direct inhibition are identical (39). There is no evidence for or against the existence of specific inhibitory collaterals or synaptic knobs. In this connection, for example, it would be very valuable to know whether dorsal root fibers of the sixth lumbar segment have knobs distributed to motoneurons with axons passing into the first sacral ventral root. The answer to such questions as this, may provide the next step in reasoning on the nature of direct inhibition. Nothing is to be gained by supposing the existence of connections, or the site on the neurons of the supposed connections, and arguing therefrom. Until it is known whether or not direct inhibition involves synaptic connection and if so where the connections are on the neurons, little advance in the understanding of direct inhibition may be expected. It would seem to be within the province of histological investigation to provide the essential data.

THE RÔLE OF EXTRASYNAPTIC INFLUENCES IN NERVOUS ORGANIZATION. One cannot doubt but that fields of current about active neurons, and chemical diffusion gradients, have an influence upon the excitability of any neurons within the radius of effective action (22, 32, 57). Stimulation of nerve fibers by other nerve fibers under certain circumstances has been known since the experiments of Hering (27). Stimulation of nerve fibers by active muscle has been known even longer (49). Somewhat in analogy with the Matteucci experiment it has

been found that the intramuscular portions of some motor and afferent fibers are excited when a muscle is activated indirectly by synchronous nerve volleys (37). Following an antidromic volley to the motoneurons a small pseudo-reflex or recurrent volley is discharged to the periphery (55, 40). The recurrent volley appears only among the motoneurons which have carried the antidromic volley, it is initiated immediately following the termination of the absolutely refractory period of the motoneuron axon, and before the electrical signs of soma activity have subsided. There is some reason to believe that the more prolonged soma activity restimulates the axon, but a full and satisfactory account of the genesis of the recurrent volley is not yet available.

The dorsal root reflex (71) is clearly the result of restimulation, by some mechanism, of the afferent fibers. At some stage in the central course of the dorsal root reflex orthodox synaptic transmission is involved for the end product possesses the characteristics of synaptically transmitted activity. Similarly, in the example of active muscle stimulating certain intramuscular nerve fibers cited above, the activity resulting in those nerve fibers reflects fully the intervention of neuromuscular transmission as one step in the chain of events (37).

It is difficult to say which and how many of the effects just described are in essence artefacts produced by synchronous stimulation or other experimental procedures. As an instance it should be noted that a certain fraction of a muscle must be synchronously active before retrograde impulses appear in the motor fibers (37). One may hold, with complete justification, that the conditions for the genesis of retrograde impulses are never achieved in the course of natural motor performance. Again, in all the experiments for which the original observations of Hering form the prototype, damage resulting in a demarcation current appears to be the indispensable factor; in other similar experiments, chemical treatment (caffeine, strychnine or veratrine) or frank electrical polarization is the essential procedure.

So far the discussion has concerned adequate restimulation of nearby excitable elements by one means or another. With much less drastic experimental manipulation it has been found that more subtle threshold effects occur in some excitable elements when activity pervades neighboring elements. Katz and Schmitt (31), Blair and Erlanger (50), and Renshaw and Therman (58) have demonstrated threshold changes in fibers consequent upon the passage of impulses in contiguous fibers. Important as these observations are for the study of nerve excitability and conduction, the influences they demonstrate can hardly be regarded as determining the prime function of a nerve fiber, viz., the transmission of an impulse from one point in the body to another.

Within the neurophil extrasynaptic actions due to fields of current and chemical diffusion gradients are of potentially greater significance. A synchronous antidromic volley in certain motoneurons is known to affect the threshold to reflex activation of neighboring motoneurons (55). It would be important to determine finally whether this action is due to recurrent collaterals or to simple current flow about the active motoneurons. When the effect demonstrated by Renshaw is inhibitory, it is particularly effective among motoneurons supplying the parts

of a single muscle. For example, stimulation of the nerve to one head of the gastrocnemius muscle inhibits reflex activity through the motoneurons of the other head. It would be interesting to know if similar alterations in reflex excitability occur when neighboring motoneurons are activated in a completely random and asynchronous manner, as for instance during the course of a static stretch reflex.

Renshaw's experiment seemingly accounts for the observation first made by Sherrington (65, 66) that simulation of one of several nerve branches to a muscle (after distal section) inhibits the innervated remainder of that muscle. In Sherrington's experiment the motor axons as well as the afferent fibers unavoidably were stimulated, and the resulting impulses in the motor axons sweeping into the central parts of the motoneurons would have had the action described by Renshaw.

It seems possible that fields of current or chemical diffusion gradients may only reach critical levels when a number of similarly orientated structures are simultaneously active. With rotation of elements, diffuse activation and 'tonic' maintained discharge, the extrasynaptic influences might approach to the steady state in character and thus play no significant rôle in phasic activity. On the other hand, it is possible that such influences would attain great significance for the long term 'state of excitability' of the nervous system. One conceives, for instance, of the possibility that long term chemical readjustments lie behind the recovery from spinal shock.

It is evident that a great deal of critical experiment and appraisal are needed before the true significance for central nervous system function of the extrasynaptic effects is understood.

CONCLUSIONS

One will readily appreciate from the preceding discussion the fact that most of the available information on functional organization relates to those systems consisting of large neurons equipped with axons of fairly high conduction velocity. In the main this is due to the technical difficulties of exploring the finer fiber systems or regions populated with small cells. This is particularly the case with respect to unmyelinated fiber systems, concerning which virtually nothing is known.

Upon occasion functional study can determine in broad outline certain details of structure, sometimes it can solve problems to which a wealth of histological endeavor has provided no definitive answer; more often it can point out to the histologist fruitful lines of enquiry and define the problem to which an answer is sought. It is a common occurrence for physiological progress to be frustrated by the paucity of histological data, but it is also true that the need for certain histological information can only be realized when the functional problem is defined. This is now particularly the case in relation to the problem of direct inhibition. There are, of course, regions where the synaptic relationships are well understood (cf. 6). More often than not, unfortunately, the regions understood from a histological point of view at the present time are not amenable to functional study. The converse is undoubtedly true.

It would be difficult to overestimate the need for factual rather than hypothetical treatment of the problems of direct inhibition as a prerequisite for adequate and complete description of functional organization, and in particular for an understanding of the means by which the spinal mechanism is fractionated by various systems of reflex and higher control.

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OBESITY

I. ENERGY METABOLISM

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Obesity is that condition in which the body contains an abnormally large amount of adipose tissue. The excessive fat may be evenly distributed or may be present to a much greater extent in some regions than in others. When the fat is sharply localized to one or more discrete encapsulated masses, one speaks of "lipomatosis" in contradistinction to "obesity."

WEIGHT. Since the accumulation of adipose tissue causes a corresponding increase in weight, much effort has been directed toward establishing a weight which is best in regard to longevity and mental and physical fitness.

The Child Health Association (1) has compiled tables that give the average weight for height and sex of large numbers of healthy children from birth through the age of 18 years. These tables are widely accepted as the most satisfactory standards.

However, Fisk (2) has shown that the average weights of persons over 30 years of age are too great as judged by life expectancy and has found that the average weight at 30 is the most desirable weight for the remainder of the life. Departures from these standards are sometimes condoned because of supposedly heavy bones, excessive muscular development or hereditary type, but according to Fisk, "Life insurance experience has shown that heavyweights, regardless of type and heredity, show an extra mortality."

Dublin and Lotka (3) have analyzed the influence of weight on the duration of life of 192,304 men aged 21 years or over when accepted for life insurance. The deaths per hundred thousand, age being disregarded, are given in table 1. Dublin and Lotka (3) concluded that "the penalty of overweight is one-fourth to three-fourths excess in mortality."

These studies become still more informative when they are related to age, since excessive weight carries a much greater risk in persons beyond 45 years of age than earlier (table 2). How great the risk is for the important years from 45 to 50 appears dramatically in table 3. It is startling to learn that a mere 25 pounds (11 kgm.) of extra weight lessens one's life expectancy by 25 per cent. Fish (2) found likewise that persons in full middle life who weigh 20 pounds (9 kgm.) more than the average instead of 10 pounds (4.5 kgm.) less are incurring an extra 25 per cent risk. In another place he pointed out that "fifty pounds overweight at age forty-five imposes as much extra mortality as valvular heart disease."

ENERGY EXCHANGE. Because of the prevalence of the belief that some patients gain weight even though they do not overeat and that others do not lose when they are underfed, there has been a continued search for some metabolic aberration in this disease.

Basal metabolism. Early students found that pound for pound obese persons produce less heat in the resting postabsorptive state than do normal controls. Had they made the comparison on the basis of height, they would have found

TABLE 1

The influence of weight on mortality; deaths per hundred thousand men accepted for insurance

WEIGHT	DEATHS
Standard	844
Underweight, total	848
Overweight, total	1,111
Underweight, 5–14%	833
Underweight, 15–34%	913
Overweight, 5–14%	1,027
Overweight, 15–24%	1,215
Overweight, 25% or more	1,472

TABLE 2

Influence of weight on mortality as modified by age. Deaths per hundred thousand

WEIGHT	AGE	
	Under 45	Over 45
Standard	463	1,308
Underweight, total	498	1,274
Overweight, total	527	1,824

TABLE 3

Influence of overweight on mortality in persons aged 45 to 50 years

POUNDS OVERWEIGHT	INCREASE IN DEATH RATE OVER AVERAGE
	per cent
10	8
20	18
30	28
40	45
50	56
60	67
70	81
90	116

that the heat production of obese persons was greater than normal. But Rubner, Lusk and others have demonstrated that the basal heat production of all mammals is proportional to the surface area of the body and that no such relation exists when either weight or height alone is used as the basis of comparison.

Benedict, using the statistical method, has published tables by which one can predict the basal metabolic rate of normal persons on the basis of age, height and weight. His values closely approximate those calculated by the surface area method. There is, then, no doubt about the range of basal heat production of normal adults.

Subsequently, Boothby and Sandiford (4) measured basal heat production in 94 obese patients and found that in 81 per cent of them the rates were within 10 per cent of the normal heat production per square meter. In 3 instances the rate fell between −16 and −20 per cent, and another patient produced heat at a rate more than 16 per cent above normal. Strouse, Wange and Dye (5) compared the basal metabolic rates of normal persons with those of subjects who were underweight and overweight. Per square meter of body surface they found practically no differences. Among 180 cases of extreme obesity Grafe (6) found only 3 in which there was a definite decrease in basal metabolic rate. The occasional moderately low rate (from −15 to −25 per cent) exhibited by an obese person does not contribute to the understanding of obesity, since equally low rates are encountered as frequently among healthy persons who are not obese. Strang and

TABLE 4

Total basal heat production of five obese women compared with ideal values

	WEIGHT	SURFACE AREA	CALORIES/SQ. M./HR.	TOTAL NUMBER OF CALORIES/HR.
	lb.	*sq. m.*		
Ideal	129	1.59	36.5	58
Observed	238	2.06	35.5	73

Evans (7) made careful measurements of the basal metabolic rates of 5 obese women, and the average values are compared in table 4 with the predictions for normal women of the same average age. The few obese persons whose basal metabolic rate is low enough to be definitely pathologic will be found to be suffering from some disease in which adiposity is a complication or an unrelated accompaniment and not the primary abnormality.

These and many other studies lend overwhelming support to the statement that obesity is not caused by lessened expenditure of energy in the basal state. In fact, an obese person produces more heat than a normal person of corresponding age, height and sex, for while both will produce the same number of calories per square meter of body surface per unit of time, the obese person has a larger surface, and therefore the total heat produced by the obese person in the basal state is greater actually than the total basal heat produced by the normal person.

Specific dynamic effect. If the heat production of a person who has been without food for twenty hours and who has been reclining quietly and comfortably for a half hour (basal state) is carefully measured and he is then fed, he will shortly produce more heat per unit of time than he did in the fasting state. This response is not caused by digestion or absorption, since it is equally great after

the intravenous injection of dextrose or some of the amino acids that result from the digestion of protein. If some metabolic fault prevented or greatly lessened this specific response to food, the person so afflicted would gain weight, provided he continued to partake of the same amount and the same kind of food and provided he did not increase his activity. His appetite might, however, direct him to eat less, since he needed less, and in that case his weight would not increase. Several students, without making any allowance for the second alternative, have attributed obesity to lessened specific dynamic effect of food.

Many attempts have been made to show that the caloric response to food is controlled by the hypophysis. Thus Plaut (8) stated, as a result of her studies, that a normal basal metabolic rate, coupled with a lowered specific dynamic response to protein, is characteristic of disease of the hypophysis. Using these criteria, she expressed the belief that she (9) was able to show that in certain cases obesity was caused by hypofunction of the pituitary. Kestner, Knipping and Liebesny subsequently published many determinations that seemed to confirm her work. The technic employed by these investigators has been criticized by a number of workers. Durr (10), for example, pointed out that a normal person exhibited no increased metabolic response to food in three hours and a response of only 18 per cent in five hours. Lauter (11) in careful studies found that the specific dynamic response of normal subjects varies so widely that only its total absence is of diagnostic significance. Gaebler (12) working in Lusk's laboratory, found that dogs from which the hypophysis had been removed responded normally to a standard protein meal.

Because of the disagreement Johnston (13) reinvestigated this question. She restricted her studies to patients in whom destructive diseases of the pituitary were demonstrated, usually by operation or at autopsy. She obtained large responses in all cases (18 to 28 per cent), and pointed out that it was not possible to secure uniform results in human beings. She found it impossible to obtain a consistent response to sucrose or aminoacetic acid in a normal subject maintained on a constant diet. This again emphasized the caution with which small responses must be interpreted.

Later work suggests that the liver is responsible for the increased heat liberated during the metabolism of protein. Bollman and Magath (14) showed that deaminization took place in the liver, and later, Wilhelmj and Mann (15) found that administration of amino acids to hepatectomized dogs did not increase the heat production. Finally Dock (16), by excluding various portions of the bodies of rats from the circulation, found that at least 85 per cent of the specific dynamic heat was liberated in the abdominal viscera.

The relation of the specific dynamic effect to the obese state has been studied so painstakingly and comprehensively by Strang and McClugage (17) that their results may be accepted with great confidence. They emphasized that the base line, that is, the value of the basal metabolism, is the most important feature of the test. Earlier workers had failed to train their subjects sufficiently. Strang and McClugage have repeated basal determinations until uniform results were obtained. In order to make a test acceptable they demanded that the basal

calories on the test day must vary less than 2 calories from the average of measurements within fourteen days. On this basis they rejected ten of their twenty-five determinations. They pointed out the fallacy of the usual way of expressing the specific effect as a percentage of the basal calories. For example, a 10 calory increase attributable to food when the subject is producing 60 calories per hour in the basal state is an increase of 16 per cent, but the same specific increment with a basal heat production of 90 calories is an increase of only 11 per cent. Since obese persons produce more heat in the basal state than do normal persons, the same absolute response to a test diet may appear to be low in obese persons when the percentile method of comparison is used. It is likewise misleading to express the response to a test diet in terms of surface area. Thus an increment of 10 calories due to food in a person whose surface area is 1.67 sq. m. gives an increase of 6 calories per square meter, whereas if the surface were 2.2 sq. m., as is common in obese persons, the increase would be only 4.5 calories per square meter. Hence, the same absolute increase in the heat results in an apparent depression for obese subjects. These workers emphasized the irregularity of the response to food when viewed from hour to hour and were in agree-

TABLE 5

Specific dynamic effect of food

TYPE OF SUBJECT	INDIVIDUAL VARIATION IN NUMBER OF CALORIES	AVERAGE NUMBER OF CALORIES
Normal	43 to 61	51
Obese	44 to 74	58
Thin	59 to 78	67

ment with Benedict and Carpenter (18) "that a true appreciation of the thermal effect of a meal is obtained by focusing the attention on the total increment." With these considerations in mind, Strang and McClugage studied 5 normal, 5 thin and 8 obese persons. The total increments for the eight hours following the ingestion of the test meal will be found in table 5.

LUXUSKONSUMPTION. Grafe (19) has maintained that in addition to the specific dynamic response, which comes to an end in twelve hours or less, the heat production of the organism throughout the twenty-four hours is influenced by the quantity of food eaten. Leaving the increases that accompany activity and the specific dynamic effect out of consideration, he expressed the belief that the intensity of the metabolism is stimulated by generous (excessive) feeding and depressed by meager supplies of food. Such a mechanism would tend to maintain constancy of weight. Obesity would develop when the mechanism failed to respond to overeating, and leanness would be the result of abnormally great responses.

Grafe and Koch (20)reported a study on a patient whose normal weight was 62.6 kgm. and whose height was 156 cm. Because of stenosis of the pylorus accompanied by persistent vomiting his weight had fallen to 40 kgm. In this

emaciated condition he produced 30 calories per square meter per hour in the resting fasting state. The prediction for a man of his age and height and of normal weight is 39 calories per square meter. Relief of the stenosis and subsequent feeding of 100 calories per kilogram brought his weight up to 60 kgm. He then produced 40.7 calories per square meter, which was within 3 per cent of the prediction for a man of his age and height and of normal weight. This study merely confirmed Schondroff's (21) and Zuntz' earlier work that starvation depressed the basal metabolism.

The reader will recall that Grafe had said that the normal organism maintains a constant weight in spite of overfeeding because the extra food stimulates the total metabolism to such an extent that the excess is oxidized, but he did not publish any data that dealt with total heat production throughout the twenty-four hours. In order to test Grafe's postulate, Wiley and Newburgh (23) studied the responses of a thin person by means of a method (24) recently developed for determining the total heat production for any desired length of time.

The subject was 28 years old, 6 feet (183 cm.) tall and weighed 45 pounds (20 kgm.) less than normal. For the first eighteen days he was fed a constant diet that yielded 2,922 calories daily. During this same period the twenty-four hourly heat production averaged 2,947 calories. His weight at the beginning was 57,562 grams, and at the end it was 57,548 grams. He was then fed a diet that yielded 4,755 calories daily. This was an increase of 1,833 calories. During the fifteen days the latter diet was taken the subject gained 4,410 grams (nearly 10 lbs.). Heat production per twenty-four hours in the second period averaged 3,082 calories. Accordingly, he produced 135 more calories when he was ingesting about 5,000 calories than when he took about 3,000 calories, an increase of 4.5 per cent in total metabolism. The increase in the surface area that accompanied the increase in weight accounted for 68 extra calories and the additional carbohydrate and fat could be expected to increase the specific dynamic effect of the second diet by 85 calories, according to a calculation by Lusk (25). Therefore the predicted increase in heat production during the second period without recourse to Luxuskonsumption is 153 calories, whereas the actual increase was only 135 calories. When the subject was partaking of the smaller diet, the fasting resting heat production was at the rate of 35.5 calories per square meter per hour. At the end of the period of overfeeding he produced 36.9 calories under the same conditions. The difference of 1.4 calories is not significant. These workers obtained no evidence that either the basal or the total metabolism was stimulated by superalimentation.

Total metabolism. It has repeatedly been observed that some obese patients who are receiving only minimal quantities of food fail to lose weight. Von Noorden (26) reported such experiences many years ago. More recently Grafe (6) has published several examples, one of which is reproduced here in part.

The patient, aged 38, was 5 feet, 4 inches (163 cm.) tall and weighed 378 pounds (171 kgm.). She became stout at 10 years of age. When she was 20 years old, she weighed 176 pounds (80 kgm.). She married at this time. Subsequently, she gave birth to 4 babies and gained weight after each delivery. When first seen by Grafe she exhibited enormous adiposity of

the trunk, legs and upper arms. During the first month of treatment she lost 30 pounds (14 kgm.). Then without change in treatment the loss of weight gradually diminished, and finally, for two weeks there was no loss at all. The weight remained stationary at 341 pounds (155 kgm.). During these two weeks her diet contained 647 calories, and the basal metabolism was 2,000 calories. Grafe calculated that the daily caloric deficit was about 2,000. In addition to the restricted diet her fluid intake was limited to about 500 cc. daily. Under these circumstances the body should have lost 8.5 pounds (4 kgm.) of adipose tissue during these two weeks. Even though the patient received four injections of mersalyl solution and 3 to 8 mgm. of thyroxin daily, the volume of urine was small, averaging 500 cc. daily. She continued the treatment at home in a little milder form and lost 13 pounds (6 kgm.) in the next few weeks.

This capacity to resist loss of weight in spite of the most rigorous treatment has given rise to much speculation and confusion. Many physicians have considered it useless or even cruel to continue to underfeed patients of this type, since they seem to be doomed to hopeless adiposity. The writers have seen many examples of this phenomenon. Such paradoxic conduct might arise from some obscure metabolic abnormality that permits conservation in the utilization of energy. In that case, the total heat production would be strikingly less than normal. But no measurement of the twenty-four hour expenditure of energy had been published prior to the studies of Newburgh and his associates. Such patients might have been studied in a respiration chamber for one or possibly a few consecutive days. But the restricted activity and short intervals of time imposed by the chamber made that method of doubtful value for this special purpose. They accordingly employed the method (23) previously cited by means of which they could measure the total loss of heat for many consecutive days while the usual activity continued. Included were subjects with various types of obesity described in the literature; that is 1, a person physically normal except for obesity who frankly admitted years of gluttony; 2, a feeble-minded girl with a low basal metabolic rate; 3, a girl with disease of the pituitary body and a basal metabolic rate 30 per cent below normal; 4, a middle-aged woman whose weight had reached 295 pounds (134 kgm.) after an operation on the hypophysis eight years earlier; 5, a young woman suffering from so-called "Dercum's disease"; 6, a middle-aged woman 5 feet 2 inches (157 cm.) tall whose weight had reached 420 pounds (191 kgm.) (menopausal obesity). In no case did they find anything unusual about the total metabolism. These patients certainly did not exhibit any capacity to live at a lesser expenditure of calories than normal persons. In fact, just the opposite was true for the patients who were active. The total expenditure was large and indicated that they produced considerably more heat than persons of the same height, age and sex whose weight was normal. The data are entirely in accord with those of Lauter (11) who found that an obese subject requires more energy to perform a given piece of work than does a normal control.

WATER BALANCE. In spite of the large expenditure of energy, periods lasting for a number of days during which the patients failed to lose weight, even though the calories of the diet were far less than the dissipation of heat, were frequently encountered. A good example of this phenomenon is illustrated by figure 1.

The patient was a young woman who weighed 275 pounds (125 kgm.) when she entered the hospital. Her basal metabolism, which was normal, amounted to 2,100 calories per twenty-four hours. Throughout the study she remained in bed. In spite of the inactivity the total twenty-four hourly heat production averaged 2800 calories. She received a diet that yielded 1600 calories daily. She was accordingly compelled to oxidize enough body tissue to produce 1200 calories daily. After a preliminary week on the diet, the analyses showed that about 150 grams of body tissue were destroyed daily. This weight times the number of days subtracted from the weight of the subject at the beginning of the

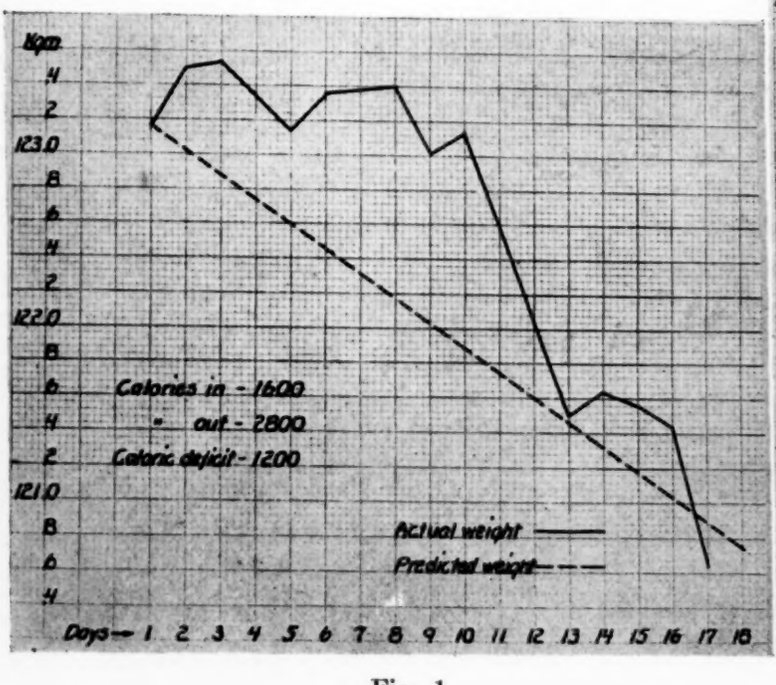

Fig. 1

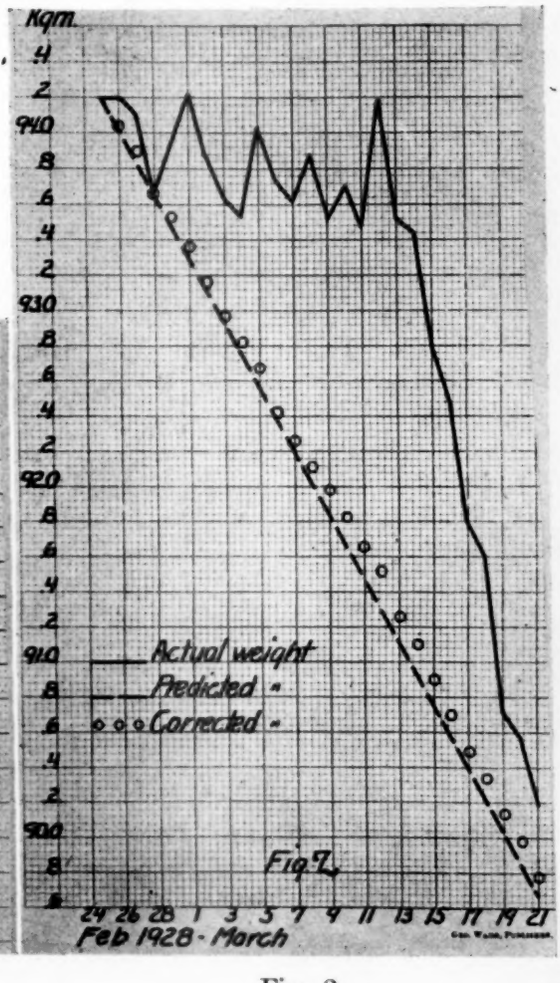

Fig. 2

experiment indicates what she would have weighed on any day if nothing else had intervened to disturb these simple relations. This hypothetic weight is represented in the diagram by the broken line. The solid line is the actual day to day weight of the patient. In spite of the underfeeding, the patient weighed approximately as much on the morning of the tenth day as she did on the first. In fact, she actually gained the first and the second day. This capacity to resist loss of weight, even though body tissues are certainly being oxidized and the end products excreted, may last two weeks or longer. But whatever the duration, it always gives way to a subsequent rapid loss of weight equal to the weight of the

tissue destroyed over the entire period of caloric restriction. In the diagram it will be seen that this patient lost as much weight in three days (tenth, eleventh and twelfth) as she was expected to lose in twelve days, due to destruction of body tissue.

It seemed to us, a priori, that this phenomenon might be caused by a retention of water subsequently followed by excretion of the excess. Accordingly, we undertook a study of the water balance (27).

In order to control our work we first studied the water relations in a normal subject while he was being underfed. For the sake of uniformity he remained in bed except when he took a few steps to the scale or the commode. To minimize error his diet was restricted to milk, sugar and water. He received 63 grams of protein, 26 grams of fat and 148 grams of carbohydrate, yielding 1,078 calories. He produced 1,688 calories daily, derived from 69 grams of protein, 91 grams of fat and 148 grams of carbohydrate (averages for each twenty-four hours). Consequently, he destroyed 6 grams of body protein and 65 grams of body fat each twenty-four hours. The combined weight of these two substances in their

TABLE 6

Concealment of destruction of tissue by retention of water

DAY OF EXPERIMENT	CHANGE IN WEIGHT OF SUBJECT	WEIGHT OF BODY TISSUE DESTROYED	WATER BALANCE
	grams	*grams*	*grams*
1	+285	95	+369
2	−225	95	−105
3	+65	95	+149
4	−125	95	−41
5	+115	95	+198
Totals.....	+115	475	+570

hydrated living state is 95 grams. It was soon noticed that this man, even though normal, failed to lose weight smoothly. Like obese persons, for several consecutive days he would lose no weight or he might even gain weight. Then his weight would suddenly decline at a rapid rate far in excess of 95 grams daily. Table 6 shows the water exchange for five consecutive days when the subject weighed 115 grams more at the end than he did at the beginning, even though he had destroyed 475 grams of body tissue. It is quite clear that the failure of the body to keep pace with the loss of tissue is attributable to the retention of water.

Since these events took place in a normal person, they are deprived of all specificity for obese subjects. The excessive retention of water is seen to be merely a by-product of undernutrition. It in no way indicates that the metabolism of obese persons is abnormal.

An analogous response by an obese girl is portrayed in figure 2. It will be noted that she weighed as much on the fifteenth day as she did at the start. Then a marked diuresis set in and continued for the next nine days, by which time she had lost an amount of weight that approximately equaled the weight

of the body tissue destroyed during the twenty-four days. The solid line in figure 2 indicates the actual weight each morning. The broken line states what she would have weighed in response to the destruction of body tissue, if the water balance had not been disturbed. The position of the circles was calculated by subtracting the weight of the extra water in her body from the actual weight. They show that her ability to resist loss of weight for more than two weeks was caused by progressive addition of water, which finally amounted to more than 6 pounds (3 kgm.).

INCREASED ABSORPTION OF FOOD. After it had been generally accepted that obese persons produce more heat than normal controls, the possibility still remained that if the digestion and absorption of food by obese persons was sufficiently more efficient than that by normal people, the former could succeed in gaining weight without overeating. Nenenschwander-Lemmer (28) has investigated this possibility. He compared the combustible materials in the feces with those in the diet. The utilization of the dietary calories, nitrogen and fat by the obese persons was 87, 84 and 83 per cent respectively. For the controls,

TABLE 7

Blood levels of fats

CONDITION OF SUBJECTS	USUAL DIET		DIET PLANNED TO INSURE UNDERNUTRITION	
	Range	Average	Range	Average
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Obese	544 to 1,117	890	446 to 802	630
Normal	371 to 1,005	591	414 to 997	607

the corresponding values were 88, 86 and 89 per cent. Obesity was not caused by an economy of food.

"LIPOPHILIA." Von Bergmann suggested that adiposity is caused by a hereditary constitutional trait of the adipose cells that enables them to accumulate excessive amounts of fat. Hetenyi (29) carried this idea one step further by postulating that fat once deposited in the depots of an obese person is prevented from leaving them and so cannot be used as fuel. Increased appetite is the natural response. To support this contention, he compared the levels of the blood fats in obese persons with those of normal persons when the usual food was taken and again after eight days of restricted diet. The data appear in table 7.

Since obese persons have more fat in the blood when food is unrestricted, they must be either storing less or mobilizing more of it than normal persons. The lowering of the blood fat level in obese persons by underfeeding is accepted by Hetenyi as evidence that they have difficulty in releasing fat from the depots in response to the increased call for energy, but it might just as well mean that fat which is being mobilized at a normal rate is being oxidized more rapidly. Later studies dealt with blood fat curves after feeding 200 cc. of cream. The average increase in the controls amounted to 84 per cent of the fasting values as compared

with an increase of only 17 per cent in the obese subjects. The individual responses, however, varied from zero to 157 per cent, and such a wide variation throws considerable doubt on the value of the averages. The lower levels in the obese subjects are offered by Hetenyi as evidence of increased avidity for fat by the depots, but since the greater metabolism of obese persons demands more fuel, why do not the lower levels of fat indicate accelerated oxidation of it?

If it were true that the adipose tissue cells of obese persons resist mobilization of fat in undernutrition, then such persons would not lose weight, or if they did, most of the loss would represent the destruction of body protein. But the following studies show that obese persons are less likely to go into negative nitrogen balance when underfed than are normal subjects. Jansen (30) placed 15 medical students whose average weight was 62 kgm. on a diet yielding 1,600 calories and 61 grams of protein daily for several weeks. Their average loss of nitrogen was 2 grams daily. Benedict's (31) 12 normal subjects receiving 1,534 calories and 51 grams of protein lost 65 grams of nitrogen from the body in three weeks. On the other hand, Keeton and Dickson (32) showed that most obese persons on a diet yielding 1,375 calories and 90 grams of protein maintained nitrogen balance, and Strang, McClugage and Evans (33) found that obese persons receiving only 440 calories and about 1 gram of protein per kilogram of ideal body weight do not lose body protein.

With these things in mind our colleague, Malcolm Block, has reinvestigated the response to underfeeding. After determining the total blood lipids during a preliminary period when the diet was unrestricted, he placed 3 normal young women and 3 obese young women on a series of diets. The first diets were arranged to yield approximately 80 per cent of the energy of the basal heat production for twenty-four hours. After seven days, blood lipids were determined again and the diets were reduced to about 60 per cent of the basal heat. Seven days later, samples of blood were again withdrawn and the calories of the diets were reduced to 40 per cent of the twenty-four hourly basal heat production. At the end of this week, samples of blood were obtained and the obese patients were all placed on a diet yielding 450 calories. The blood fat readings are recorded in table 8. An examination of these values indicates that they rose and then fell both in the obese patients and in the controls, and it is not possible to detect any significant difference in the responses of the two groups to underfeeding. In addition, Block calculated the dissipation of heat from the insensible loss of weight (24) and estimated the caloric deficit by comparing the energy of the diet with the expenditure of energy. The differences were then converted to weight of body tissue destroyed, on the assumption that it consisted entirely of fat. The results for the 3 obese patients are found in table 9, which also records the daily intake of nitrogen and the urinary nitrogen. It will be seen that each of the patients lost almost exactly the amount of weight that she was expected to lose if the body tissue destroyed to make up the caloric deficit was solely adipose tissue. Furthermore, the comparison between the dietary and the urinary nitrogen shows that the patients lost only negligible amounts of body protein.

All of these later studies indicate that obese persons release fat from the stores

TABLE 8

Variation in total blood lipids in three normal and three obese young women on various ditse

DIET	BLOOD LEVELS OF FATS	
	Normal controls	Obese subjects
	mgm./100 cc.	*mgm./100 cc.*
Unrestricted	499	700
	523	540
	610	560
80 per cent of basal calories	706	808
	750	575
	710	700
60 per cent of basal calories	846	868
	843	700
	806	850
40 per cent of basal calories	703	910
	605	850
	542	610
20 per cent of basal calories		710
		910
		608

TABLE 9

Loss of weight and daily dietary intake and urinary output of nitrogen in three obese subjects

NUMBER OF SUBJECT	DAILY LOSS OF WEIGHT		DAILY NITROGEN	
	Predicted	Actual	Intake in diet	Output in urine
	grams	*grams*	*grams*	*grams*
13	254.6	255.4	13.81	14.34
14	250.7	249.2	13.81	13.35
15	222.8	222.8	13.66	13.75

TABLE 10

Respiratory quotients after a test meal

	HOURS AFTER MEALS					
	1	2	3	4	6	8
Normal subjects	838	810	804	823	812	819
Obese subjects	746	772	752	740	761	795

as a source of energy as readily as normal persons do. In fact, it seems likely that the obese persons do this more readily, since they usually remain in nitrogen balance when they are underfed, whereas persons of normal weight do not.

The fasting respiratory quotients in obese persons are lower than they are in normal controls. According to the usual interpretation, this means that the former persons are oxidizing more fat. Quotients published by Strang (34) are enlightening. The fasting values obtained from 7 obese patients ranged from 0.698 to 0.830 and averaged 0.757. The controls under similar conditions ranged from 0.770 to 0.848 and averaged 0.783. These same persons were studied again after a test meal consisting of 40 grams of protein, 26 grams of fat and 52 grams of carbohydrate. Quotients were obtained repeatedly after the meal for eight hours and are presented in table 10.

Since both the quotients obtained during fasting and those obtained after the ingestion of food are lower in obese subjects, we have before us classic evidence that such persons are metabolizing more fat than the normal controls. They cannot at the same time be storing more fat or withholding more of it in the depots.

SUMMARY

These many painstaking investigations of the metabolism of obese persons have failed to disclose any abnormal process that accounts for the accumulation of the fat. On the contrary, they have demonstrted that obese persons produce more heat in the basal state, that they expend more energy to perform a measured amount of work and that their total heat production is greater than that of normal persons of similar age, height and sex under the same circumstances. Since they are unable to absorb more energy from their food, they must eat more than normal people simply to avoid loss of weight.

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II. ETIOLOGICAL ASPECTS

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The foregoing presentation of the various aspects of energy exchange leave no retreat from the commitment that all obesity, in the final analysis, is the result of an overall inflow of energy which has exceeded the overall outflow, with a resultant retention in the body of the excess energy as fat. The question of the fundamental etiology of obesity, therefore, must concern itself not with whether but with why there occurs a positive energy balance. Are there various mechanisms which may be responsible for the initiation and continuation of positive energy balance? An answer supported by unquestioned experimental evidence as regards the human being cannot now be given, but the various possibilities that exist will be set forth in the succeeding paragraphs. The fact remains, in any case that, regardless of the mechanism involved in its production, all obesity (clinical as well as experimental) responds with a predictable loss of body fat to measures which reduce energy intake below energy expenditure.

Many of the hypothetical mechanisms alleged to result in the production of obesity are nebulous and devoid of experimental confirmation. An attempt will be made to review those concepts which in the author's opinion deserve consideration. Since it is taken for granted that the body fat does not arise spontaneously, and that every gram of body fat had an isocaloric precursor which entered the body from without, we shall omit those concepts which insist that the human body has learned to outwit the fundamental laws of thermodynamics.

Finally, with regard to thermodynamics, a brief definition of terminology is necessary in order to provide proper insight. When one speaks solely in terms of energy equilibria, he is indicating the *end result* of a balance sheet in which

the only considerations involved are total caloric intake, total caloric output and change in body weight (taking into consideration water exchange for the period). From this point of view all obesity is necessarily of one kind, that is, a positive energy balance phenomenon. This is indisputable. But in the sense that changes in caloric intake, caloric expenditure or both might be influenced, directly or indirectly, by psychic, hereditary, endocrine or metabolic aberrations and by functional or organic changes in the central nervous system, various types of obesity are possible. It is with these considerations in mind that we proceed to examine the evidence.

HYPOTHALAMIC OBESITY. Rather intriguing and recently more clearly defined is the experimental production of obesity in normal animals in which bilateral lesions of the hypothalamus have been produced. Beginning with Erdheim (1) in 1904 many clinical reports have appeared suggesting that destructive lesions involving the floor of the third ventricle and either including or sparing the hypophysis have resulted in marked obesity. Working with dogs Camus and Roussy (2, 3) followed by Baily and Bremer (4) and later by Keller and Noble (5) noted the development of obesity following experimental injury to the posterior hypothalamus. The hypophysis was carefully avoided and it was concluded that the obesity produced was the result of the hypothalamic lesion. The recent work in dogs by Heinbecker and White (6) appears to delineate with greater specificity the hypothalamic area involved. They found that lesions of the posterior hypothalamus, caudal to the paraventricular nuclei, which interrupt fibers whose cell bodies originate in the caudal portions of the paraventricular nuclei (resulting in retrograde degeneration of these structures) result in obesity; that similar lesions not producing the retrograde degeneration in this region did not produce obesity. They noted, too, that the coexisting loss of the cells of the supraoptic nuclei (producing diabetes insipidus) resulted in the development of a greater degree of obesity (75 per cent to 110 per cent increase in weight in 6 months as compared with 50 per cent for the paraventricular nuclear lesion alone). They conclude that the specific obesity lesion in the dog is a diminution in the number of cells in the caudal portion of the paraventricular nuclei; that in the presence of such a lesion a simultaneous decrease in posterior pituitary secretion intensifies the resulting obesity.

In a recent report, Brooks, Lambert and Bard (7) indicate the development of obesity in six of twelve monkeys (Macaca mulatta) following experimentally produced hypothalamic lesions. Mature monkeys more than doubled their weight in 8 to 10 months, after which the weights remained relatively constant. The presence or absence of an associated diabetes insipidus or hypogonadism did not influence the development of obesity. The total caloric intake of the obese monkeys was much greater than that of their non-obese controls.

Much more extensive observations on this type of obesity have been made in the rat. Smith (8, 9) had shown in 1927 that extreme obesity could be produced in rats by hypothalamic lesions placed so as to avoid the hypophysis. By means of the Horsley-Clark stereotactic instrument fairly standard hypothalamic lesions are now produced and obesity develops with great regularity.

From a series of experiments by Hetherington and Ranson (10, 11, 12) the following conclusions can be drawn: 1, destruction of the hypophysis of the rat does not result in the production of obesity; 2, large, symmetrically placed lesions of the hypothalamus produce obesity in virtually 100 per cent of rats so treated; 3, obesity produced by bilateral hypothalamic lesions are not dependent upon intact nervous pathways between the hypothalamus and the hyphophysis nor upon the presence or absence of the hyphophysis. They believe as do Heinbecker and White (6) (the latter used dogs) that the obesity is dependent upon the interruption of descending fibers which leave the ventromedial hypothathalmic nuclei and normally descend toward the brain stem.

A few studies of the metabolism of animals made to become obese by experimentally produced hypothalamic lesions have been made. As mentioned above, Brooks et al. (7) noted a greatly increased caloric intake in their operated monkeys. When deprived of food the obese monkeys lost weight slightly less rapidly than did the non-obese monkeys. Hetherington and Ranson (11) studied the spontaneous activity and food intake of rats with hypothalamic lesions. Two things were clear. 1. All of the operated animals were much less active than their controls. 2. Most of the animals ate a great deal more than their controls. Because some of the animals became obese without eating more than their controls the authors seemed unduly troubled. The lowered activity could easily account for the apparent discrepancy. This decreased activity probably explains why Brooks' obese monkeys (7) failed to lose weight as rapidly as their controls when both were deprived of food.

The excellent work of Long and his associates (13–18) upon metabolism in hypothalamic obesity may be summarized as follows: 1, shortly after the hypothalamic lesions were placed, the animals began to consume 2 to 3 times the amount of food eaten by their littermate controls and became extremely obese; 2, when paired-fed with their controls, only 1 of 10 operated animals outgained its control; 3, studies of basal and total oxygen consumption and of basal and postprandial R. Q.'s in paired-fed operated (non obese) and obese-operated rats showed no deviations from the normal, which were not fully accountable on the basis of increased body weight and the eating habits of the operated animals; 4, "the development of obesity is apparently a consequence of increased appetite, and is not associated with any fundamental disturbance in metabolism."

Thus, it appears that in at least three mammalian species (rat, dog and monkey) experimentally produced destructive lesions of the hypothalamus which sever descending nervous pathways from the parventricular nuclei lead to the rapid development of obesity. Clinical examples of this phenomenon, although not as well studied or as easily controlled, may be found in the literature. An adequate explanation for the resulting obesity is found in the tremendously increased food intake and to a lesser degree in the concomitant decrease in spontaneous activity exhibited by experimental animals. That there has occurred no significant change in fundamental metabolic processes may be adduced from the facts 1, that operated animals paired-fed with their

controls behave, with regard to gain in weight, like the controls; 2, that operated animals deprived of food lose weight at the expected rate, any slight deviation probably being accountable on the basis of decreased activity of the operated group, and 3, that oxygen consumption and R. Q. studies on such animals reveal no deviations from the normal.

The conclusion seems irresistable that dysfunction of nervous elements which originate in or are mediated through certain nuclei of the hypothalamus is capable of producing a tremendous increase in the desire for food. By what means normal function of this system controls the hunger mechanism or how its destruction releases the inhibitory control remain for the present unknown. That an organic mechanism exists is evident. That this mechanism plays a significant rôle in the etiology of human obesity is not evident. That it cannot be disregarded, however, as a possible factor in human obesity is equally clear.

ENDOCRINE OBESITY. 1. *Effect of endocrine function upon energy balance.* It has gradually become apparent that one cannot think in terms of hyperfunction or hypofunction of any one of the endocrine glands without, at the same time, considering the effect of the specific dysfunction upon the remaining glands of internal secretion. In this sense most endocrine disturbances are polyglandular, even though replacement therapy for a primary hypofunction may result in return of the whole system to normal. It seems reasonable to state that until a specific metabolic aberration which can lead to obesity is demonstrably related to a given endocrine dysfunction it is premature to attach significance, from an etiological point of view, to such time-worn expressions as pituitary obesity, gonadal obesity, etc. It is true that certain non-specific metabolic changes such as hypoglycemia or reduced basal heat production may be instrumental in stimulating increased intake of energy or in decreasing energy expenditure. But such metabolic changes may result from a variety of types of endocrine imbalance. When obesity does occur in association with some such metabolic change it would be more realistic to term it "low energy output obesity" in the hypometabolism case and "high energy intake obesity" in the hypoglycemia case, both cases evidencing a positive energy balance To say "hypothyroid obesity" implies that hypothyroidism per se produces obesity. As a matter of fact, most patients suffering from the severest form of hypothyroidism, myxedema, do not become obese (19). They automatically adjust caloric intake downward in accordance with the decreased need for energy.

One can analyze, in a similar way, the example of hypoglycemia. The following provides an interesting comparison of two such patients upon whom we (20) have recently concluded prolonged metabolic studies.

1. Each had been suffering for several years from daily attacks of severe and prolonged hypoglycemia due to pancreatic islet cell tumors. Blood sugar levels during attacks varied in each between 10 and 40 mgm. per cent and the fasting blood sugar value was almost always below 50 mgm. per cent.

2 One had maintained a normal weight throughout his illness, hunger not having been a prominent symptom of his hypoglycemia. The other had gained 50 pounds since the onset

of her illness, 20 pounds of which had been added in the past year. She had found that ingestion of food delayed or alleviated attacks.

3. Each successful surgical removal of the insuloma restored carbohydrate metabolism to normal. Each, however, *maintained* his pre-operative weight, i.e., the obese woman remained obese despite removal of the initiating cause of her positive energy balance.

4. Because of her obesity a reduction diet was prescribed. She lost 30 pounds but soon her desire for more food overcame her desire to lose more weight and she gradually gained back what she had previously lost.

It is evident that the metabolic change occasioned by the abnormal endocrine function resulted in obesity only when it caused increased ingestion of food; that removal of the metabolic aberration did not result in loss of weight; and that the food habits, acquired during the hypoglycemic period, persisted as a cause of obesity after removal of the initiating influence.

One is obliged, therefore, to interpret the endocrine element in the production of obesity in terms of its effect upon total exchange of energy, remembering that the same dysfunction may not affect the energy balance of two individuals in the same way. The endocrine dyscrasia is not the thing that produces accumulation of adipose tissue.

2. *Effect of endocrine function upon distribution of body fat.* Superficial observation alone is sufficient to convince one that the distribution of body fat is significantly influenced by the endocrine status of the individual. Patients previously normal suddenly begin to redistribute body fat in rather constant patterns soon after the onset of certain endocrine disorders. A classical example is found in the case of Cushing's syndrome in which obesity of the face and neck become prominent along with loss of subcutaneous fat from the extremities. When positive energy balance exists and there occurs an actual increase in total body weight, the added fat is distributed, for the most part, in the upper half of the body. The total body weight, on the other hand, may be normal or subnormal and yet the obesity of the face and neck is sufficient to suggest that the patient is greatly overweight (see figs. 1 and 2).

Similarly, all who have observed the remarkable change that occurs in a sexually mature female who develops a virilizing tumor of an ovary, must agree that whatever may be the hereditary gene pattern for specific bodily form, the influence of normal or abnormal endocrine function is an important determinant. One is all the more convinced when normal female distribution of fat once again returns upon removal of the ovarian neoplasm.

No one doubts that two perfectly normal individuals, one male and one female, will become obese if fed 1000 calories in excess of their daily requirements for a sufficiently long period. If this is done at age 6, the distribution of deposited fat will be general and almost identical. If carried out after sexual maturity, on the other hand, the localities of fat distribution will be significantly different. The influence upon regional distribution of fat has been exerted by a difference in endocrine function.[1]

[1] "*Regional lipophilia.*" Much has been written, but little is known concerning the local mechanisms which allow one region of the body to become a fat depot while preventing such accumulation of fat in another. It is interesting to remark in passing, however, that in

Thus, with regard to the endocrine influence upon obesity, two factors must be sharply distinguished: (1) the influence of the disturbance in increasing food intake and/or decreasing energy expenditure; (2) its influence in determining regional distribution of fat. The former is much less important than the latter. That is, individuals with a given endocrine lesion may or may not be obese. But obese individuals possessing certain endocrine abnormalities are likely to exhibit a fairly characteristic distribution of body fat. The examining physician must, therefore, pay close attention to the distribution of fat in any obese individual. It will occasionally lead him to the *additional diagnosis* of a specific endocrine disease.

Fig. 1

Fig. 2

Hypophysis. 1. *Pituitary obesity.* This once popular clinical diagnosis is fortunately gradually disappearing. It was used to attach an etiological tag to the previously described syndrome of adiposo-genital dystrophy. This syndrome of obesity and hypogonadism sometimes seen in patients who have destructive lesions in the region of the stalk of the pituitary gland is reproducible in animals by means of experimental lesions at the base of the brain close to pituitary infundibulum (see hypothalamic obesity). That production of this complex is independent of the presence or absence of the pituitary gland has been demonstrated (8, 12). It appears from the evidence at hand that clean removal of the pituitary (experimentally) or destructive lesions of the gland (clinically), which do not injure important adjacent hypothalamic nuclei,

humans in whom an abnormal distribution of fat can be etiologically related to a known endocrine dysfunction the steriod hormones, adrenal cortex or gonads, appear almost invariably to be involved.

produce progressive emaciation and cachexia (8, 21, 22) and that dysfunction of hypothalamic centers is required for the development of adiposo-genital dystrophy. The mechanism by which genital dystrophy results from hypothalamic injury when the pituitary gland remains intact needs further study.

It is to be recalled that the thyroid and adrenal cortex which also depend for their integrity upon intact pituitary function, are not anatomically disturbed by the experimental lesion which produces adiposo-genital dystrophy (8). Hypophysectomy, as is well known, results in hypoplasia of all three (thyroid, adrenal cortex and gonads).

Rony (23) and Schwartz et al. (24) have reported that the administration of gonadotropic substances to patients with adiposo-genital dystrophy has no effect upon the adiposity even though it does induce genital development. One is forced to conclude from the available information that in true adiposo-genital dystrophy the increased desire for food (and consequently the obesity) is the direct result of hypothalamic dysfunction; that the accompanying genital dystrophy may involve a specific effect of the hypothalamic lesion upon the gonadotropic function of the anterior pituitary gland; or that the obesity per se has affected genital development. In the latter connection, the frequent occurrence of delayed puberty in obese youngsters needs further emphasis (see under Gonads).

2. *Cushing's syndrome.* This special situation has been discussed in some detail above. From a metabolic point of view it would appear that the interesting clinical picture is produced by prolonged increased secretory activity of the adrenal cortex (25). That in some cases the lesion is primary in the adrenal cortex is clear (26). That an indistinguishable syndrome occurs in association with basophilic adenomas of the pituitary gland has also been shown (27). The connecting link is found in the fact that a large percentage of the latter group of cases have developed hypertrophy of the adrenal cortices (28). Since Cushing's syndrome, even when secondary to "pituitary basophilism", appears to exist by virtue of hyperadrenocorticism, there is no reason to incriminate the pituitary gland for any one of the many characteristic manifestations of the disease.

Adrenal cortex. A characteristic type of distribution of body fat occurs with great regularity in patients with Cushing's syndrome. As indicated above the syndrome has been interpreted as being due to hyperadrenocorticism (25). The peculiar distribution of fat has attracted comment of virtually all who have studied these patients. The characteristic appearance has led to the beautifully descriptive term of "humpty-dumpty disease". We (29) have recently conducted a prolonged metabolic investigation of such a patient and present his appearance as a typical example (figs. 1 and 2). The striking accumulation of fat about the face, neck, shoulders and upper chest has been noted almost invariably.[2] Contrary to expectations from outward appearances, however,

[2] An opportunity was afforded to demonstrate that this tissue is actually fat. An abscess developed in the subcutaneous tissue beneath the chin. At the time of incision for drainage, a thick mass of adipose tissue was found to extend as far as the probing finger could reach.

there is very little adipose tissue under the abdominal skin. The protruding abdomen appears to be due to loss of tone of the abdominal musculature. Shortening of the thoracic spine due to collapse of vertebral bodies (where this has occurred) intensifies the appearance of obesity of the trunk. Thus, the total amount of subcutaneous adipose tissue and, in fact, the total weight of the patient, may be below average. But, overweight or not, the amount of fat about the face, neck and shoulders is excessive.[1] Loss of body weight by caloric restriction proceeds normally (30). The basal metabolic rate, though frequently low, is variable.

A metabolic aberration in the form of excessive glyconeogenesis from protein with a resultant unavailability of amino acids for replacement of essential tissue needs (all due to hypersecretion of adrenal steroids) has been hypothesized by Albright (25) to account for many of the physical abnormalities observed in such patients. He refers to Woodyatt's finding (25) of negative nitrogen balance despite high protein feeding in an acute case of Cushing's syndrome. Although insufficiently confirmed in patients with this disease, the thesis finds support in the animal experiments of Long and his associates (31). If true in Cushing's syndrome, it is conceivable that the increased appetite observed in the early stages of the disease represents an unsuccessful attempt at replacement of essential body protein and that despite the continued loss of body protein a positive *energy balance* exists with a resultant deposition of body fat. That a dissociation of protein and energy balance may occur in the opposite direction, i.e., a negative energy balance associated with positive nitrogen balance has been shown in our laboratory (32).

Pancreatic islets. A relationship between hypoglycemia and the hunger mechanism has long been recognized. It has been suggested that a mild degree of hypoglycemia, insufficient to produce typical symptoms, could be the cause of the desire of obese persons for increased amounts of food. Studies of blood sugar levels in obese people fail to disclose any increased tendency toward hypoglycemia. In fact, the trend is decidedly toward hyperglycemia (33).

Chronic, recurrent, spontaneous hypoglycemia in humans, severe enough to produce symptoms, may result from any one of a variety of causes (34). Obesity, however, is a prominent feature in only one of these disturbances, namely, organic hyperinsulinism. In this condition hypoglycemia is likely to be more severe and more prolonged than in any of the other conditions which result in low levels of blood sugar. The patient often learns that he can prevent or alleviate attacks by frequent ingestion of food. Hence, some become severely obese. But others with hypoglycemia of equal severity never experience an increased desire for food and do not become obese.

Functional hyperinsulinism (34) represents a very frequent type of spontaneous hypoglycemia manifesting itself in the form of transient symptoms occurring two to four hours postprandially. These individuals invariably maintain a normal level of fasting blood sugar. No lesion of the islet cells is recognizable in this group, but it is suggested that functional hyper-responsiveness of the insulogenic mechanism accounts for the severe but transient hypoglycemia

demonstrable. These individuals are of average weight or lean; are highstrung, emotionally unstable and display many other signs of instability of the vegetative nervous system. We have studied a large number of such cases and are impressed by the virtual absence of obesity in this group.

It appears, then, that if hypoglycemia is to play a significant rôle in stimulating excessive ingestion of food, it must be severe and prolonged to the point of easy clinical recognition. That such a situation is absent in the great mass of obese individuals is obvious.

These clinical observations are borne out by the animal experiments of McKay and Calloway (35) and of Barnes and Keeton (36). It was found that rats could be made to ingest sufficient extra food to produce obesity only when severe hypoglycemia was maintained continuously by the use of large amounts of protamine zinc insulin. Rapidly absorbed insulin which produced severe but relatively transient hypoglycemia did not result in increased total consumption of food. It is, therefore, extremely doubtful that "relative hyperinsulinism" plays any part in the production of obesity, with the rare exception of the patient harboring a pancreatic insuloma.

The possibility that insulin may stimulate conversion of carbohydrate to fat has been suggested on several occasions. But even were this assumption proven to be true, such a metabolic process would have no influence upon the production of obesity. For whether energy requirements are provided by ingested carbohydrate or by fat derived from ingested carbohydrate is inconsequential. Caloric balance would remain the same in either case and further assumptions would be necessary in order to explain increase in weight.

Finally, any possible rôle, in the etiology of obesity, of lipotropic substances such as choline and methionine (37) or the pancreatic lipocaic of Dragstedt (38) is beyond present knowledge and need only be mentioned as being related to the metabolism of fat.

Thyroid. Little need be said regarding the relationship of hypothyroidism to the development of obesity. It is clear to all that if the basal heat production is reduced by 40 per cent and, in addition, the daily spontaneous activity is diminished by the very nature of the disease, the total dissipation of energy and, consequently, the requirement for energy will be significantly reduced. Such an individual will unquestionably gain weight if he continues to consume an amount of food which was formerly barely sufficient to maintain his weight. Interestingly enough, however, and contrary to the usual clinical teaching, obesity is by no means a reliable index or a constant feature of hypothyroidism (19, 39, 40). For the majority of such patients the hunger mechanism does an excellent job of diminishing food intake to the newly acquired level of metabolism. In those in whom such adjustment is less sensitive, either obesity or undernutrition may result.

Gonads. The response of the body weight to gonadectomy is extremely variable in animals and in humans. Castration of domestic fowl has long been known to produce larger and fatter animals. Castrate lambs and goats gain less weight than their controls (41, 42) Gonadectomized male or female rats

show no increase in body fat over their controls (43). In men castration results in obesity in some and in the "tall, lean castrate" in others. Women are said to gain weight rapidly after the menopause. That this is not true is well shown in the statistics compiled by Rony (44).

From a practical point of view obesity in association with hypogonadism occurs with greatest frequency in pre-adolescent boys and girls. At this stage of life hypogonadism is normal and the obesity is correctly regarded as "exogenous". The advent of normal puberty and the development of normal secondary sex characteristics is again correctly taken to mean that the continued obesity is not related to gonadal function. But pity the child who is fat and in whom the onset of puberty is delayed for several years! He is labelled as a case of "gonadal obesity", "pituitary obesity" or "adiposo-genital dystrophy" and placed in a category of endocrine cripples. Fortunately, the erroneous diagnosis proves to be shortlived. The majority of such patients sooner or later pass spontaneously through a delayed puberty period and become sexually mature. The attendant redistribution of body fat may change the appearance to one which conforms more closely to the normal male or female but the excess weight remains unless the patient is underfed. As mentioned above, the use of gonadotropic substances in such cases may promote sexual maturity, but does not affect excess weight (23, 24).

On the other hand, it has been our experience in these children that simple weight reduction by dietary means has resulted in the fairly prompt establishment of puberty. This interesting phenomenon, i.e., the rôle of obesity per se in influencing gonadal function in adults as well as in adolescents requires elucidation.

Heredity versus environment. Little in the way of positive information can be cited for the case of "hereditary obesity" in humans. As stated earlier, the search for the fundamental cause or causes of obesity must no longer concern itself with whether the development of obesity is associated with an excessive intake of calories. That part of the problem is settled! Obesity, regardless of any precipitating cause, cannot develop unless associated with a positive energy balance. But the underlying mechanisms which drive the individual to ingest energy in excess of his needs remain elusive. Does there exist a gene or gene complex which, through its influence upon the central nervous system or the endocrine system or by another mechanism, forces upon the stigmatized individual an irresistible desire for excess calories? The answer to this important question is not yet available.

Statistical studies (45, 46) while in many respects suggestive of an hereditary fault, have not eliminated environmental influences as factors of prime importance in the acquisition of obesity as a familial characteristic. Studies upon groups of identical twins simply emphasize the importance of environmental influences upon body weight. The studies of Verchuer (47) and of Neuman (48) indicate that the average variation of body weight of identical twins is two to three times greater than the average variation of any of the other anthropologic measurements. That the average variation in body weight of fraternal twins

is greater than that of identical twins (48) gives no information referable to the transmission of a gene capable of producing the *abnormal state* known as obesity.

The important experiments of Danforth (49) proved conclusively that in a yellow strain of wild mice there exists a gene, probably associated with that imparting the yellow color, which regularly produces obesity; that the obesity begins after sexual maturity; and that the obesity is much more marked in the female than in the male harboring the same gene. Experiments with this strain carried out in our laboratory, indicate that an increased consumption of food occurs in those aninals which become obese. Thus, in this species, there can be little doubt that heredity plays a dominant rôle in initiating a positive energy balance. That a similar background may exist in some humans can at present be neither denied nor affirmed.

On the other hand, the positive influence of environmental factors upon body weight is well established. Almost all students of obesity agree upon one point, i.e., that at least some obese individuals are victims of circumstance and that their obesity is the result solely of a perverted appetite. Hence, all classifications of obesity include a type designated as "exogenous obesity". The experienced clinician, while justifiably reserving the right to consider hereditary factors in the etiology of obesity, has no doubt about the influence of early training, acquired habits of food selection and emotion upon the development of the condition. Appetite, as opposed to hunger, is a product of one's environment and is importantly affected by pleasurable and painful experiences. One emotionally disturbed person is distressed by the sight or smell of food (anorexia nervosa) while another finds relief of mental anguish in the pleasurable sensation of a full stomach. The direction of his reaction toward food may be significantly influenced by past experiences, training and habit. This is not to imply that all or even a majority of obese people are emotionally upset. Many are simply victims of early training. They have been conditioned to a higher "satiety level" than is required by the physiological needs of the body.

Anomalies of metabolism. A number of theories have been proposed suggesting an abnormal metabolic pathway for fat, protein or carbohydrate, the result of which was to lead to the deposition and retention of excessive amounts of adipose tissue. Devoid of experimental confirmation, such concepts arose because of the common clinical observation that many obese people subjected to stringent and controlled underfeeding fail to lose weight as expected. The experiments dealing with the masking effect of temporary retention of water during periods of actual destruction of tissue (see Part I) soon dampened the enthusiasm of those who had contended that the fundamental laws of thermodynamics could be violated by certain fat people. The more recent concepts of disordered metabolism as a cause of obesity have included the obvious necessity for excessive calories The abnormal metabolic process is then held responsible for the excessive desire for food. Since some of these alleged metabolic aberrations have been dealt with in detail elsewhere the reader is referred to the previous discussions where possible.

1. *Hypoglycemia* (See under Endocrine Obesity).
2. *Lipophilia* (See under Lipophilia, Part I).

An additional comment regarding lipophilia (fat-fixation, fat immobilization, etc.) is indicated at this point. Bauer (50) although holding firmly to the concept of increased lipophilia as the important mechanism in the production of obesity, freely admits that restriction of calories is the only sensible and effective method of treatment. The fact that any obese individual when fed a diet submaintenance in calories, but sufficiently adequate in protein to provide for nitrogen equilibrium, loses 50, 100 or more pounds of body weight, is in itself the strongest argument against immobilization of body fat as a source of fuel. One cannot reconcile the concept of tissue lipophilia with the finding that body fat will provide the energy to make up whatever caloric deficit is provided by underfeeding.

3. *Hypolipemia.* Wilder (51) has suggested that a slightly greater than normal withdrawal of fat from the circulation postprandially could explain the delayed sense of satiety encountered in obese people and could account for the increased total intake of food. Such a mechanism, if it existed, could explain the *maintenance* of obesity (once established) were the increased rate of withdrawal of circulating blood fat due to the increased need for energy known to be associated with the obese state. But in order to be important as a *cause* of obesity, such an abnormality would have to be shown to exist in certain individuals before the onset of obesity, and to persist after successful dietary reduction of weight. Such information is not available. A point, therefore, to be evaluated with great care in interpreting any metabolic variant observed in obesity, is the effect of the obesity per se in producing the abnormality encountered.

Static obesity. Finally, any discussion of possible etiological factors involved in the production of obesity should bring out several significant points with regard to the maintenance of the obese state. A critical analysis of static obesity helps to simplify the problem of its production.

It is common knowledge that most obese people eventually reach a level of body weight which remains relatively constant thereafter. The level may be 200 pounds or 500 pounds or any other amount which indicates the presence of obesity. What are the energy relationships when this static weight is reached? Does the cessation of gain in weight mean that the individual is now eating less than he has been previously? Decidedly not! Assuming that his gain of weight has been steady and even, he will now be found to be eating at least as much as he was at any time during the period of increasing weight. The only possibility remaining is that he is now expending more energy than had been the case during the time that his weight was increasing. Let us examine the facts.

That basal heat production rises in relation to increasing surface area is a classical and well established phenomenon. This means that as the body enlarges, the requirement for ingested energy to make it grow larger increases progressively. There comes a time when, because of increased size of the body an equilibrium is established between caloric inflow and caloric expenditure, and

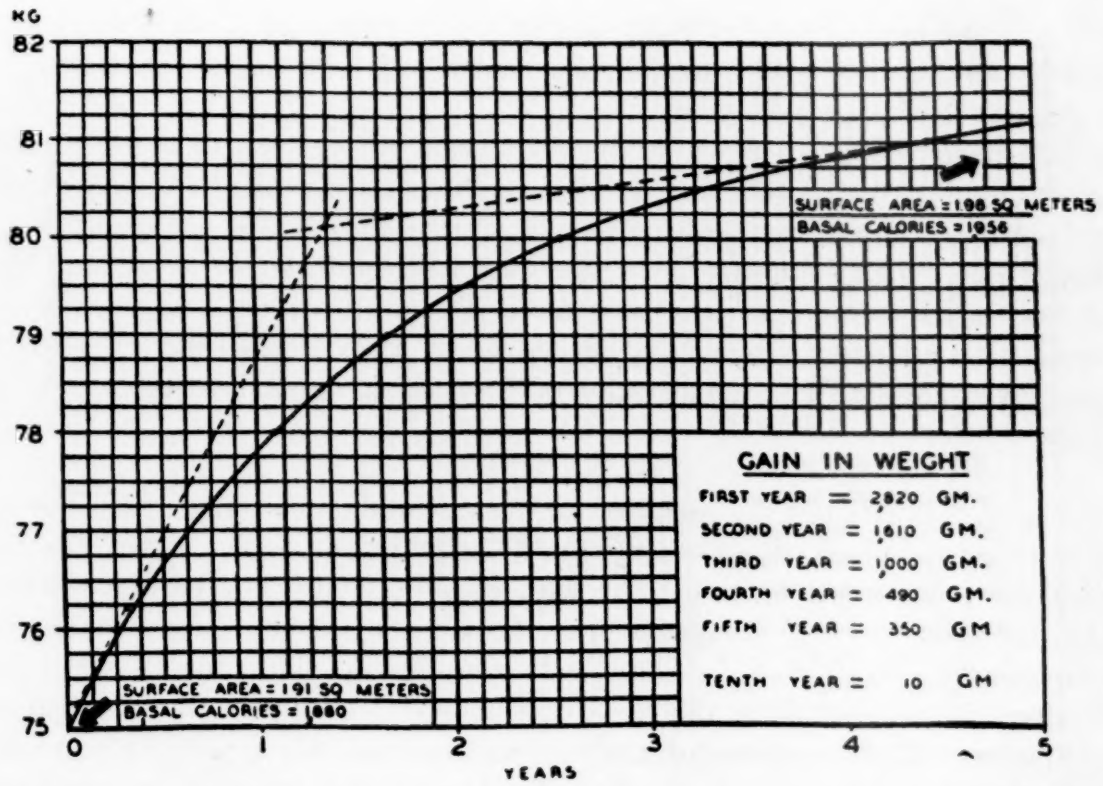

Fig. 3. Weight plotted against time, with an initial dietary excess of 80 calories per day. The broken lines indicate the rate at which weight would be added in the first and fifth years.

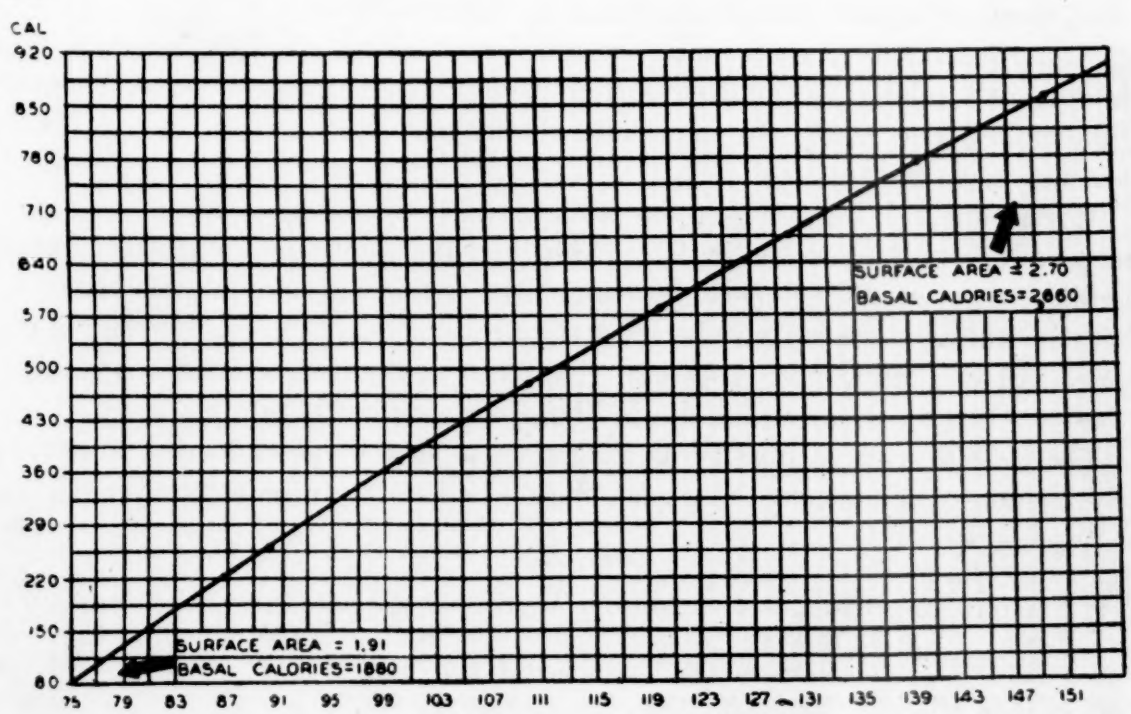

Fig. 4. Excess calories plotted against weight. In order to maintain a given rate of weight gain caloric intake must be increased progressively as the body enlarges.

positive energy balance ceases. Weight is then maintained at the new high level unless energy intake is further increased. The amount of energy now

required for maintenance of body weight was formerly sufficient to produce at first a rapid and later a slow gain in weight. Figures 3 and 4 are clearly illustrative of this phenomenon.

This physiological increase in the dissipation of energy as surface area increases is not peculiar to any class of warm blooded animals (52). It occurs in any normal animal or human who is forced-fed to the point of increased surface area. Thus the mechanisms for the *dissipation* of energy in the obese individual respond in the normal way. One is faced, therefore, with the inevitable conclusion that the defect in obesity, whatever its dynamic mechanism, is to be found on intake side, i.e., the ingestion of excessive amounts of energy.

SUMMARY

Obesity is invariably the result of an overall intake of energy which has exceeded the total dissipation of energy by the body, i.e., a positive energy balance. With the few exceptions associated with an abnormally low basal metabolic rate (and the latter is by no means limited to fat people), the mechanisms for the dissipation of energy from the obese body are normal. The fundamental fault, therefore, is to be found in the cause or causes of an excessive desire for food.

Experimentally a number of mechanisms leading to a desire for excessive amounts of food have been demonstrated. They include bilateral hypothalamic injury (rat, dog and monkey), severe and prolonged insulin hypoglycemia (rat), and hereditary bulimia in a strain of mice. It has not been established that these are significant causal factors in human obesity.

Abnormalities of the endocrine system which affect adipose tissue are more importantly related to the distribution of body fat than to excessive accumulation of it. The search for metabolic aberrations which could lead to obesity has not been fruitful.

Environmental influences upon appetite are established causes of a very large number of obese patients. These include emotional difficulties, family habit, early training, affluence, boredom, etc. Other patients become obese, not because they eat more, but because they expend less. Various types of prolonged illness, advancing years and change to less active occupations result in obesity when a corresponding diminution in appetite fails to take place.

The classification of obesity as exogenous, endogenous, endocrine, pituitary, thyrogenous, gonadal, etc., is misleading and should be discontinued.

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THE CELLULAR COMPOSITION OF NORMAL BONE MARROW AS OBTAINED BY STERNAL PUNCTURE

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A critical appraisal of the literature on the cellular constitution of the marrow of healthy individuals can be made only in the light of an understanding of the anatomy and physiology of the marrow, the objectives of marrow studies, the methods of study available, and the many variables affecting the composition of the specimen obtained for examination.

ANATOMY AND PHYSIOLOGY OF BONE MARROW (1). The marrow is the largest, most widely dispersed, and least homogeneous organ in the body. Its volume is one to two times that of the liver, or 1,600 to 3,000 cc. in the adult. Its location within the interstices of cancellous bone and between the trabeculae of the marrow cavities in long bones makes complete study of all the marrow an almost impossible feat. The vascular and nerve supply are imperfectly understood, what knowledge we do have being based on studies on birds or lower mammals under abnormal conditions. These studies suggest that sinusoids probably substitute for the capillary bed of most organs and that these sinusoids are probably a closed system with most of the sinusoids collapsed at any one time. Human bone marrow varies from an almost exclusively fatty tissue to the most cellular red marrow, with all gradations in between. While in the adult fat predominates in the shafts of the long bones and red marrow in the cancellous heads of these bones and the flat bones, all gradations are often seen in a single histologic section of grossly red marrow from a healthy person. Not only is cellular marrow interspersed with fat but in the cellular area adjacent foci consist of different cell types and lie next to vascular channels filled with blood. In disease this patchy structure is still more pronounced, as witness the lesions of multiple myeloma, metastatic neoplasms, and the lipoid histiocytoses.

The important functions of the marrow are the formation of the cells of the blood, the removal of particulate matter from the blood, and the possible production of antibodies and plasma proteins. Except in disease or embryonic life all formation of cells of the erythrocytic, granulocytic, and thrombocytic series apparently occurs in the marrow, and the formation of cells of the lymphocytic, monocytic, and plasmacytic series is shared with the lymph nodes, spleen, and other lymphoid or reticuloendothelial tissue. Evidence suggests that erythrocyte formation is primarily intravascular in temporarily closed sinusoids and that the other cells are formed extravascularly.

The formation of these cells involves the fundamental processes of growth: increase in size or hypertrophy, either mitotic or amitotic cell division or hyperplasia, maturation or differentiation, and, in addition, release into the circulation. In the formation of platelets the splitting off of fragments of cytoplasm is also important. The important cells vary in diameter from one or two micra for the smallest platelets to sixty micra or more for the megalokaryocytes.

OBJECTIVES OF OBTAINING MARROW SPECIMENS. Marrow specimens are studied to learn more about its structure and its function in health; that is, to add to knowledge of normal anatomy and physiology, to detect deviations from normal in disease for diagnostic purposes, or to obtain material for tissue culture, for bacterial culture, or for identification of parasites. The method of choice depends upon the objective.

METHODS OF OBTAINING MARROW SPECIMENS, WITH ADVANTAGES AND DISADVANTAGES OF EACH METHOD. The ideal method of obtaining marrow specimens would permit removal of a large, representative, measured specimen in such form that accurate cell identification and counts of each type of cell may be obtained and all structural relationships could be observed. Unfortunately, no such method exists. The methods which are available include postmortem sections, procurement of a button of marrow by use of a trephine during life, and aspiration of marrow through a needle, either postmortem or during life, and, in conjunction with the latter method, the possible removal of a core of marrow by the use of a serrated hollow drill longer than the needle (2). Each method has certain advantages and certain disadvantages.

Postmortem study of marrow is probably best for determining the normal anatomy of the marrow. It permits sections of all the major bones, so that a gross determination of the relative distribution of yellow and red marrow may be made. Gross inspection of the cross sections of bone may guide the selection of the tissue to be removed for microscopic study. Numerous enough and large enough specimens may be obtained to discount the patchy distribution of cells within the marrow. As with the other methods, fixed sections, imprints, and cell suspensions may all be studied, and special injection technics through the vascular system may be employed. There are, however, many disadvantages to postmortem studies. A diagnosis made then is obviously too late to be of value to the patient. Unless the specimens are obtained within a few minutes after death autolysis will have set in (3), cell morphology is destroyed, and coagulation of the blood prevents aspiration of a satisfactory sample. Only rarely is postmortem material available within a few minutes of death when there is adequate evidence of the previous good health of the subject. The few studies so far made (4) on such material have been inadequate in one or more respects. Not enough subjects have been examined. Criteria of previous health have been inadequate. Gross and microscopic sections have not included enough bones. Imprints and cell suspensions have usually not been made. Yet it appears that accurate data on the actual normal anatomy of human marrow can only be obtained by postmortem study. It is important when such a study is made that measured or weighed blocks of marrow of one or more cubic centimeters volume be suspended in measured volumes of homologous serum and that total nucleated cell counts as well as differential cell counts on smears from this material be compared with aspirated marrow from an adjacent area and with sections and imprint preparations. The volume and morphology of the unsuspendable residue should also be noted.

Obtaining a button of marrow by trephine during life (5) has the advantages of permitting the ready preparation of imprints and microscopic sections. It has

several serious disadvantages, however. It leaves a disfiguring scar which is especially objectionable to women. It is a more formidable procedure than aspiration. So small an area is examined that patchy lesions are easily missed. A total nucleated cell count and an accurate differential cell count are impossible, as will be shown later. Although Dameshek, Henstall and Valentine (6), from their study of the comparative quality of preparations made from material obtained by trephination and by sternal aspiration from the same patient, concluded that the cellularity, reticulocyte percentage, relative number of erythroblastic to granulocytic cells, and absolute number of early nucleated cells in smears made from material obtained by trephination was much greater, Falconer and Leonard (7), in a comparable study, after evaluation of preparations made by simultaneous trephination and sternal aspiration on 13 cases state: "In only one of these were significantly superior preparations as to number, type, and distribution of cells obtained by the trephine method."

Needle aspiration of marrow has, since the introduction of the method by Caronia (8) in 1922 from the tibia in children and by Arinkin (9) in 1927 from the sternum in adults, shown such definite advantages for diagnostic purposes that it is now used all over the world. It is a simple procedure which can be done in the office or at the bedside. Cell morphology is better than with any other method. Total nucleated cell counts as well as differential cell counts are possible. Enough material is obtainable for bacterial (10) and tissue (11) culture, peroxidase and reticulocyte stains, thick drop preparations, or other special procedures, and to permit diagnosis of patchy lesions such as multiple myeloma and lipoid histiocytoses, as well as the diffuse lesions of leukemias, anemias, marrow aplasia, malaria, and leishmaniasis. If imprints and sections are desired small fragments of marrow may usually be fished from the punctate from which satisfactory preparations may be made (12), or a hollow drill with serrated edge (2) may be inserted through the needle to obtain a core of marrow before aspiration. The disadvantages of the method are that patchy lesions will occasionally be missed and that there may occasionally be failures to obtain marrow. One of the authors (E. E. O.) has failed to obtain marrow only once in the last thousand punctures, but marrow was obtained from this patient on another occasion. There were 5 failures among the first 62 sternal punctures done (13). It seems certain, however, that failure to obtain marrow would result in cases of osteosclerosis. The criticism often raised (14) that cell relationships are lost and that a variable amount of blood is aspirated is valid if only the suspension is examined, but as pointed out above material suitable for sections and imprints can usually be obtained. In the majority of instances aspirated material is satisfactory for diagnosis, if a diagnosis can be made by examination of the marrow obtained by any technic, although x-ray studies and necropsy marrow examination are unquestionably more likely to detect osteomyelitis and metastases of malignant tumors.

THE RELATIVE MERITS OF SECTIONS, IMPRINTS, AND SMEARS FROM CELL SUSPENSIONS. Sections, imprints, or smears from cell suspensions can be made from material obtained by any of the above methods.

Microscopic sections have the advantage of preserving cell relationships and of including all types of cells. Advocates of this method claim to be able to identify cells in such sections, and some (4) even report differential cell counts on sections. However, even if all the cells and cut fragments of cells could be identified in a section, which the authors doubt, differential cell counts would be grossly in error, for the reason that the larger the cell the more times fragments of such cells would appear in the section, and the smaller the cell the more likely is it to be obscured by an overlying cell or fragment of cell. When the finest nuclear detail is often necessary for identification, how can the cell fragments from which most or all of the nucleus has been cut off be identified, and how is one to decide how large a fragment of a cell must appear in a section for it to be counted?

Imprint or touch preparations made from the freshly obtained buttons of marrow obtained by trephine during life or within a few minutes after death have the advantages of giving excellent cell morphology in the thinner areas and of preserving the general grouping of cells in the marrow. The inclusion of cell fragments and the understaining of thicker masses of cells which adhere to the slide make it impossible, however, to do a systematic differential cell count of all cells in the imprint, and the probable differences in the adhesiveness of various types of cells make it very unlikely that the proportions of cells adherent to the slide actually represent the proportions of cells in the marrow. Furthermore, because of the tendency of similar cell series to appear in groups in the marrow an accurate differential cell count is impossible, even if all cells were identifiable, unless extremely large numbers were counted, because of the chance selection of patches of cells of predominantly one type.

Cell suspensions obtained by the technic of sternal marrow aspiration have the great advantage that they may be thoroughly shaken, insuring a uniform distribution of the cells so obtained, of permitting quantitative total nucleated cell counts which give a rough idea of the degree of cellularity of the marrow, and of permitting accurate cell identification and differential cell counting. The proportion of mechanically traumatized or damaged cells is much less than in the other methods. The disadvantages are that cell relationships are lost, the cells may not be equally easily aspirated so that the proportions of cells may not be that actually occurring in the marrow, and that the amount of dilution with blood and plasma may not be accurately determinable. The fat cells, fibroblasts, and reticuloendothelial cells known to be present in the marrow are not usually present in the aspirated material in identifiable forms. That these disadvantages are not serious is demonstrated by the fact that correct diagnoses are usually achieved by examination of such material. The cell relationships which existed in the marrow can usually be accurately predicted from the cell types found in the punctate, the relative percentages, and the total nucleated cell count, if in the interpretation the existant knowledge of the gross and microscopic cellular distribution in the corresponding disease is considered: for example, the finding of Gaucher cells, tumor cells or a moderate increase in plasmacytes indicates the presence of the patchy lesions of Gaucher's disease, metastatic malignant tumor, or multiple myeloma; whereas, the great increase in the type cell of any

form of leukemia or the immature nucleated erythrocytes indicates the diffuse hyperplasia characteristic of the corresponding leukemia or anemia of the variety indicated by the cell morphology. The nucleated cell count shows a high correlation with the proportion of fatty or fibrous tissue to hematopoietic cells, so that low total nucleated cell counts indicate aplasia, agranulocytosis, or osteosclerosis. Occasionally, in certain aleukemic leukemias and when the needle enters the metastasis of a malignant tumor, the cells are so firmly adherent to one another that a representative number is not aspirated and the total nucleated cell count is low. Usually, however, a sufficient number of the characteristic cells of aleukemic leukemia is aspirated to establish the correct diagnosis, and occasionally this is true in malignant tumors. In the presence of metastases of malignant neoplasms, other methods are more reliable than any form of marrow examination. A high nucleated cell count indicates hyperplasia, except in those cases when a very small quantity of marrow is aspirated and the tip of the needle lies in a nest of cells or when the proportionately larger evaporation in relation to the small volume is not prevented. These possibilities are eliminated by taking a larger amount. The variable dilution with blood and plasma applies also to sections and imprints. It must be remembered that blood is present in the sinusoids and vascular channels in both normal and pathologic marrows, and is of necessity included in such preparations as well as in preparations made by the technic of aspiration.

It is evident, therefore, that the method of choice for clinical diagnosis or for obtaining marrow for bacterial or tissue culture is the aspiration and adequate examination of sternal marrow; and that for the study of the normal anatomy the method of choice is the procurement and adequate examination of fresh necropsy material from healthy persons who were executed or killed accidentally or in battle.

THE SITE OF PUNCTURE. It is possible to obtain marrow by needle puncture aspiration from many different bones, including the shaft or head of the tibia, the head of the femur, the crest of the ilium, the body or manubrium of the sternum, the ribs, and, according to Nordenson (15), the vertebrae. Since in adults it is known that the marrow from the shafts of the long bones is predominantly fatty it would seem desirable not to use this site in a study of the normal marrow picture. In infants and young children this marrow is deeply red, and considerable work has been published on the tibial marrow in children (16). Obviously, any study of marrow obtained by biopsy should state the exact site of puncture. A number of different sites have been used by different authors. There is considerable evidence that the marrow obtained by aspiration from different areas in the same bones or from different bones in the same person is of similar composition, if the person is healthy and the sites are those that normally show red marrow. Nordenson (15) compared the differential cell counts on sternal marrow with those taken from the vertebra, rib, pelvic bone, and tibial epiphysis in two patients with normal peripheral blood pictures and found good agreement in all the areas except the latter which was almost completely inactive. Stasney and Higgins (17) compared the marrow obtained within five hours after accidental death from 14

persons without evidence of disease of any hematopoietic organ or signs of infection. They examined sternal marrow, rib marrow, and vertebral marrow and found excellent agreement in the proportions of the granulocytic and nucleated erythrocytic series and in the differential cell counts. Yamamoto (18), Lossen (19) and Williams (20) each reported that there was a marked similarity in the cellular pattern of marrow taken from different regions. Conclusions to the contrary by Helpap (21) were based largely on gross pathologic findings and not on actual comparative cell counts.

Since the majority of the work has been done on the sternum and since it is the most convenient site accessible for puncture it would seem best to standardize on the sternum as the site of choice, except when local tumors demonstrated by physical examination or radiography indicate a reason for puncture at another site.

Greif (22), comparing different sites of puncture in the sternum, found excellent agreement between neighboring punctures at the same horizontal level and not quite as good agreement between punctures at different vertical levels, although as will be mentioned later his method may introduce considerable error. A number of authors prefer the manubrium to the body of the sternum. Veeneklaas (23), from anatomical studies of fifty sternums in infants, recommended the manubrium of the sternum as the site of choice for puncture in this age group. Diwany (24), from x-ray and postmortem studies, also recommends that the manubrium be used for the first few months but prefers the first sternebra in infants over six months of age.

Since sternal puncture is more easily performed through the sternomanubrial junction because the soft cartilaginous end of the bone is easily penetrated by rotating the needle with very little pressure, obviating the use of a hammer or mallet to drive in the needle, and since this would standardize the level of puncture, it is recommended that the midline of the sternomanubrial junction should be the site of choice for introduction of the needle. It makes little difference whether it is directed into the body of the sternum or into the manubrium. This choice may be based on the anatomic structure of the particular subject. The body of the sternum is usually preferable in adults and the manubrium in children under six months of age or in those adults whose manubria project anterior to the surface of the body of the sternum.

THE TECHNIC OF PUNCTURE. Many different methods of sternal puncture have been described, but it is not in the province of this article to review these methods. Certainly, however, an article on normal values should state the exact method employed. The methods vary in the size of the needle, the direction in which it is inserted, the site of puncture, and particularly in the method of forcing the needle into the marrow cavity. One investigator (25) used a mallet or hammer to drive in the needle. Often a guard which is supposed to prevent too deep penetration by the needle is utilized (26). While any of the methods described is apparently satisfactory as far as obtaining material suitable for diagnosis is concerned, it would seem that the simplest and safest technic producing the least discomfort for the patient should be the method of choice. The simplest and

safest method (27) so far available seems to us to be that one in which, after adequate local anesthesia of the skin and periosteum, a 16 to 18 gauge needle with a well fitted stylet and a knurled head is introduced diagonally through the sternomanubrial junction and rotated without much pressure in one direction like a drill, at all times under perfect control by the fingers of the other hand resting on the sternum. It certainly requires the least complicated and expensive apparatus.

THE AMOUNT OF MATERIAL NECESSARY. The amount of material aspirated obviously will have a considerable effect on the number of marrow-specific cells and on the distance from the needle from which they can be obtained, yet some authors fail to mention the amount of marrow aspirated (28). Greif (29) found that if 0.1 cc. portions were aspirated successively and examined separately, whether the first 0.1 cc. was aspirated quickly and the second slowly or vice versa, the first 0.1 cc. had a consistently higher total nucleated cell count than the second 0.1 cc. He, therefore, recommended that the first 0.1 cc. be used for examination. In 1938 (22) he aspirated and made total nucleated cell counts on successive drops up to 5 drops on 15 cases. A consistent tendency for the total nucleated cell count to decrease in the first three drops and then to level off was noted. He also compared the first 0.1 cc. with the second 0.1 cc. aspirated on 15 cases and noted a lower count in the latter than in the former. Segerdahl (30) compared successive 0.2 cc. aspirations and found a considerable decrease in count in the second 0.2 cc. Reich and Kolb (31), on marrows from patients with various diseases, compared the first 2.5 cc. aspirated with the second 2.5 cc. aspirated. Inspection of their figures reveals no consistent tendency for the second count to be lower or higher than the first count, and the majority of the counts agreed within the limits of error of cell counting technic, although the authors themselves conclude that "statistical analysis indicates that quantitative determinations on aspirated marrow samples are inaccurate." One of us (E.E.O.) has made a few unpublished studies, comparing the first 0.1 cc., the first 1 cc., and the last 1 cc. of 10 cc. samples aspirated. A considerably higher count was noted in the first 0.1 cc. than in the 1 cc. sample, but the first 1 cc. and the last 1 cc. of 10 cc. aspirated showed comparatively good agreement, considering the unavoidable errors in cell counting technic. There was no consistent tendency for the last 1 cc. to have a lower count than the first 1 cc.

On the basis of the higher counts in the first 0.1 cc., many authors have felt that it was wiser to do all the work with the first 0.1 cc. aspirated from the marrow (32). However, for practical clinical purposes there are several objections to this. It is difficult to measure accurately such a small volume, and the fact that there is great change in cell counts in successive 0.1 cc. portions aspirated means that considerable errors will be produced from this cause alone. Removal of such a small quantity can not possibly aspirate cells from more than a few millimeters from the tip of the needle; whereas, if 1 cc. or more is aspirated cells from a considerable distance might be included, thus obtaining type cells from patchy lesions which the needle did not actually penetrate. During the process of transfer of a small volume, such as 0.1 cc., to a watch glass

mixing, and reaspiration for counts and making smears, very appreciable loss by evaporation must take place, and this will be variable with the time consumed and the temperature and humidity of the room. Since, when larger volumes are aspirated the count no longer becomes progressively lower but tends to remain uniform or show plus or minus variations, it is obvious that one is not continuing to draw only blood and further dilute the sample but to withdraw a more uniform mixture of blood and marrow. Inspection of table 1, in which total nucleated cell counts are summarized, also reveals that where larger amounts were taken the total variability of range was markedly decreased. Part of the variability when small samples are taken is undoubtedly due to the chance location of the tip of the needle near a nest of cellular marrow or within a blood sinus or in a less cellular fatty area. The larger samples also permit use of a measured amount of anticoagulant in relation to the amount of marrow fluid aspirated and examination over a period of time with a chance to do additional tests, such as a peroxidase stain, multiple smears, a reticulocyte count

TABLE 1

Total nucleated cell counts per cubic millimeter of sternal marrow in adults

AUTHOR	NO. OF CASES	CC. ASPIRATED	AVERAGE	RANGE
Tempka and Braun (28)	3		55,200	
Segerdahl (30)	110	0.2	77,194	10,600–238,000
Napier and Gupta (63)	10	2.0	53,500	32,500–116,000
de Renzi and Fuortes (28)	20		33,115	
Pitts and Packham (64)	24	2.0	28,100	7,700– 46,000
Gormsen (65)	30	0.1–0.2	64,000	18,000–216,000

etc., should the routine total nucleated cell count and differential cell count indicate that such additional examinations are necessary.

It is recommended, therefore, that for diagnostic purposes 1.0 cc. or more of marrow be aspirated and that if the actual cellular constitution of marrow as it exists is to be determined, fragments of marrow, after measurement of the volume, be suspended in a known volume of homologous serum and counts be made on these or from a core of material removed with a hollow drill with a serrated edge and similarly suspended. If the smaller quantities are to be used for diagnostic purposes it will certainly be necessary for the hematologist always to remove exactly the same amount of marrow in the same manner, to make a correction for evaporation based on the temperature, humidity, and time of making preparations from that marrow, and to determine his own normals for that particular technic.

PREPARATION OF MARROW FOR EXAMINATION. Great variations in the methods of preparing marrow for examination exist. Some authors examine smears or imprints of solid fragments of marrow (33). Some allow the material to clot and make tissue sections (34). Others centrifugate and make smears and sections from the buffy coat (35). A few, including Greif (22), Joppich and

Liessens (36) and Rohr (37), place the aspirated material in a watch glass and dip filter paper in it with the implication that the filter paper removes the blood and leaves the marrow, although no evidence for this rather astounding view is found. Some use no anticoagulant, depending on the speed of manipulations to prevent clotting, others have used a constant volume of isotonic anticoagulant, and others have used a standard amount of dry potassium oxalate for the volume of marrow obtained. Still others (34) have depended on paraffined glassware and heparin for prevention of coagulation. Henning and Korth (38) injected 0.5 cc. of sodium citrate solution into the marrow cavity and assumed that the citrate in the same volume as injected is reaspirated, although Tocantins, O'Neill and Price (39) have shown that a 500 cc. transfusion can be given by injection through a sternal puncture needle, and one of the authors (E. E. O.) has given as much as 1,500 cc. consecutively through the same sternal puncture needle. The procedures that have been done on aspirated marrow include smears on cover slips or slides stained with Leishman's, Giemsa's, May-Grunwald-Giemsa's or Wright's stain, with or without the use of buffer phosphate solution, supravital

TABLE 2

Total nucleated cell counts per cubic millimeter of sternal marrow in children

AUTHOR	NO. OF CASES	AGE	CC. ASPIRATED	AVERAGE	RANGE
Vogel and Bassen (56)	41	1 day–12 years	0.1–0.2	300,000–400,000	50,000–1,000,000
Diwany (24)	5	Infants	0.1–0.2		280,000– 700,000
	10	3.5–5.5 years	0.1–0.2		75,000– 160,000
Shapiro and Bassen (66)	35	Birth to 24 hrs.	0.1–0.2	185,843	
		7 days	0.1–0.2	134,200	

preparations, reticulocyte stains, peroxidase stains, total nucleated cell counts, bacterial cultures, tissue cultures, hemoglobin determinations, red cell counts, and smears, imprints, or sections of marrow fragments or buffy coats. Others (35) have simply measured the thickness of the buffy coat as the criterion of the cellularity of the marrow.

On the basis of experience gained in the examination of marrow from well over 1,000 sternal punctures, it is recommended (27, 47) that 1.0 cc. or more of marrow be aspirated, introduced immediately into a test tube containing 2 mgm. of dry potassium oxalate per cubic centimeter of marrow, tightly corked, and thoroughly shaken immediately. On this material a minimum examination should include a total nucleated cell count by the technic of leukocyte counting, the making of thin smears which are to be stained with Wright's stain and buffer phosphate, and, if a general survey of the slide does not immediately give the diagnosis, a differential cell count of at least 500 cells in the thin area of the slide where the red cells do not touch each other. A general survey of the slide at a magnification of 200 times, with oil covering the slide but not touching the objective lens, is a most valuable procedure, since it permits survey of

thousands of cells. Frequently, peroxidase stains, reticulocyte counts or bacteriologic cultures are indicated. When malaria or leishmaniasis (40) is suspected and the organisms are not found in thin smears of marrow a careful survey for the presence of parasites in thick drop marrow preparations is suggested.

SELECTION OF SUBJECTS. Any article purporting to give the data on marrow of normal individuals should state the number, age, sex, race, criteria of health, geographic locality where the study was made, the altitude, and the general nutritional status of the subjects examined, as well as indicate whether the women studied were pregnant or nonpregnant and, in the case of the latter, the relationship of the time of obtaining the marrow to the menstrual cycle. No article gave all these data. Some (41) omitted data essential for their inclusion in this discussion, while others had to be eliminated because the authors (42) specifically stated that their "normal" subjects were patients in Outpatient Clinics or hospitals with a variety of diseases, including tuberculosis, catarrhal jaundice, or simply patients with unspecified diagnoses with "normal blood counts." Such data are of value, but not as normal standards. The minimum requirement of health would seem to be that the patient feels himself to be perfectly well, has had a physical examination, routine urinalysis, and routine blood examination including the sedimentation rate, and has no history of recent illness. It might be permissible to include patients with hernia, hyperplastic adenoids or tonsils, or similar anatomic deviations who are awaiting operation (43), but results obtained by examination of patients ill of various diseases not specifically affecting the blood-forming organs should not be used as normals. One of the most quoted series of "normal values", that of Nordenson (15), fails to include a single healthy person. As has been previously pointed out (44), there have been erroneous "normal values" for the cell counts and hemoglobin content of the blood in the literature for years because of failure of investigators to observe these criteria of healthy persons.

CRITERIA OF CELL IDENTIFICATION. Since in hematologic literature so many different names have been used for the same cell and so many different cells have been described under the same name it is essential that the terminology used in each paper be accurately defined or that a reference be given in which the criteria of cell identification used are accurately described. Extreme difficulty was encountered in preparing the tables for this article because of the widely different terminology used, even in this small series of cases (table 3.) Many authors (45) failed to state their criteria of identification, and several (46) who did give accurate descriptions of the cells used names for them identical with those used by others for cells which were entirely different. In preparing the tables the authors were forced to group the cells from the descriptions given as nearly as possible in corresponding categories. However, because of the inadequate criteria given in some papers, undoubtedly errors were made, and many of the original authors may disagree with the groupings made here.

We, therefore, recommend that after the war, in order to obviate this difficulty, an international committee of hematologists, physiologists, anatomists, histolog-

TABLE 3

Differential cell counts reported on sternal marrow of healthy individuals

AUTHOR	DATE	NO. OF CASES	AMOUNT OF MARROW	GRANULOBLASTS	PROGRANULOCYTES A	PROGRANULOCYTES S	N. GRANULOCYTES	N. METAGRANULOCYTES	N. RHABDOCYTES	N. LOBOCYTES	EOSINOPHILS	BASOPHILS	MONOCYTES	LYMPHOCYTES	PLASMACYTES	KARYOBLASTS	PROKARYOCYTES	KARYOCYTES	METAKARYOCYTES	MISCELLANEOUS
			cc.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*							
Holmes and Broun (67)	1933	7		2.4[a]	(		7.0[f])	6.7[i]	14.0[k]	17.4[o]	1.0	0.3	9.0	24.9[p]	0.0	2.6[r]	(	5.2[s])	6.9	0.0
Young and Osgood (13)	1935	28	2.0	0.4[a]	1.5[c]	1.7[e]	0.8[f]	7.4[g]	24.1[k]	13.3	1.4	0.2	2.1	10.6		1.7[s]	1.5[x]	7.0[x]	5.0[x]	20.8[A]
				0.0–1.2	0.0–3.8	0.0–4.0	0.0–2.6	1.8–9.8	15.8–33.0	7.4–25.2	0.0–3.6	0.0–0.8	0.0–4.2	4.8–16.0		0.0–4.2	0.0–5.0	2.0–15.0	2.0–10.0	12.8–31.8
Reich (26)	1935	1	10.0	2.0[a]	(		20.0[f])	5.0[j]	10.0[k]	25.0[m]	1.0	0.0	1.0	10.0	1.0	2.0	(	8.0)	15.0	0.0
Segerdahl (30)	1935	110	0.2	1.26[a]	1.51[c]	(	14.94[f])	15.25	8.92[k]	21.73[m]	2.96	0.16	2.09	18.4	0.43	(			12.28[u])	0.08[B]
Suarez (68)	1936	2	4.5–5.0	2.0[a]	(		20.0[f])	5.0[j]	10.0[k]	25.0[m]	1.0	0.0	1.0	10.0	1.0	2.0[t]	(	8.0[u])	15.0[x]	0.0
Rohr (37)	1936	10		1.3[a]	3.8[d]	(	12.3[f])	7.9[g]	40.9[k]	17.2[n]	3.7	0.4	1.5	11.0	1.8	(			30.1[u])	5.2[C]
				0.2–2.6	1.4–8.4			2.8–11.0	35.0–50.6	11.4–29.2	1.8–7.0	0.0–1.0	0.0–1.4	1.6–19.0	1.0–3.6					
Vogel, Erf and Rosenthal (69)	1937	21	0.1–2.0	1.6[a]	(		21.5[f])	(	30.2[l])	34.0[n]	2.1	0.07	0.0	8.6	0.0	0.14[s]	(	7.1[u])	22.6[x]	3.55[D]
				0.4–5.0			12.0–32.0		18.0–40.0	20.0–50.0		0.0–1.0		1.0–19.0		0.0–1.4		1.2–23.0	7.0–60.0	
Napier and Gupta (63)	1938	10	2.0	1.2[a]	0.7[c]	(	4.4[f])	9.7[h]	25.3[k]	16.8[n]	9.1	0.2	3.0[o]	6.25	0.6	0.7[s]	3.4[u]	(	21.6[x])	0.0
				0.4–1.5	0.0–1.5		1.0–9.5	6.4–15.0	14.0–38.5	6.5–25.6		0.0–1.0	1.2–7.0	3.0–12.0	0.0–1.6	0.0–1.5	0.4–10.0		16.0–25.5	
De Renzi and Fuortes (28)	1938	20		0.69[b]	(	4.4[e,e])	15.71[f]	(19.15[g]	)	22.45[m]	5.38	0.66	0.41	4.2	0.0	1.27[s]	(		25.0)	0.86[E]
Henning and Keilhack (70)	1939	10	0.5	0.60[a]	(2.2[c,e]	)	7.9[f]	6.3[g]	34.3[k]	8.2[n]	6.8	0.1	0.7	2.7	0.1	1.8[t]	(	6.4[v])	21.5[x]	2.7[F]
				0.2–1.2	1.4–3.0		2.6–11.2	5.0–10.8	18.4–47.8	2.0–11.0		0.0–0.2	0.2–1.4	0.6–5.6	0.0–0.4	0.8–3.6		4.7–8.0	13.8–28.8	
Scott (32)	1939	6	0.1	1.77[a]	4.5[c]	(	13.05[f]	15.7[j]	16.0[k]	14.75[n]	3.5	0.1	0.80	10.85	0.87	0.43[w]			17.8[x])	0.0
				0.8–3.2	2.4–6.8		10.0–16.3	12.8–17.6	12.4–17.6	13.1–16.4		0.0–0.4	0.4–1.2	6.4–13.9	0.4–2.0	0.2–0.8			14.8–19.2	
Pitts and Packham (64)	1939	24	2.0	0.3	1.0	0.5	6.0	5.6	23.7	16.6	1.8	0.3	1.4	16.8	0.0	0.1	2.5	5.0	1.9	16.2[A]
				0.0–0.8	0.1–2.2	0.0–1.4	1.4–11.0	1.4–9.4	12.4–34.2	10.4–31.0	0.0–7.4	0.0–0.8	0.0–3.5	9.2–30.4		0.0–0.4	0.6–4.0	1.3–8.0	0.4–3.2	10.5–24.0
Morrison and Samwick (71)	1939	28	1–2 drops	1.5[a]	(		24.0[f])	6.0[g]	8.25[k]	34.0[n]	0.75	0.0	0.0	7.5	1.5	(		2.35[u])	11.13[x]	3.0[G]
Gormsen (65)	1940	30	0.1–0.2	1.0[b]	(3.0[c,e]	)	14.0[f]	17.0[g]	6.0[k]	18.0[n]	3.2	0.03	1.0	18.0	0.8	(		10.0[u])	5.0[x]	2.85[H]
Hansen (43)	1941	68	0.2	3.0[a]	3.6[c]	(	4.6[g])	(32.7[g,k]	)	16.6[m]	2.8	0.1	1.3	18.0	0.5	1.6[x]	(		15.0)	0.1[I]
				0.8–8.0	0.3–6.8		2.1–8.8	19.1-44.8		5.9–36.3			0.0–3.9	10.4–29.1	0.0–1.7	0.0–3.5			4.2–30.4	0.0–0.4
Jacobsen (43)	1941	75	0.5	1.17[a]	2.43[c]	(	18.76[f])	18.11[g]	7.40[k]	12.21[n]	2.87	0.18	1.18	13.9	0.53	0.86[t]	(	5.81[u]	12.06[x]	1.38[J]
				0.75–1.9	1.0–4.15		12.0–24.35	8.75–27.35	2.6–14.45	5.6–28.95			0.5–2.95	6.85–25.05	0.05–2.0	0.5–1.6		1.8–12.2	7.4–19.9	
Farber (72)	1941	25	0.3	1.26	1.0	(	7.65)	(4.06	)	38.18	1.9	0.18	3.69	11.95	0.82	2.44	(		19.10	8.63
Weighted means of 473 cases				1.53	14.45			24.07		20.04	2.86	0.15	1.71	14.20	0.51	17.21				2.80
Range of means				0.4–3.0	4.0–24.0			4.06–48.8		8.2–38.18	0.75–9.1	0.0–0.66	0.0–9.0	2.7–24.9	0.0–1.8	9.5–30.1				0.0–20.8
Weighted means of the individual series				63.10									1.71	14.20	0.51	17.21				2.80

[a] Myeloblast, [b] hemocytoblast, [c] premyelocyte or progranulocyte A with azurophil granules, [d] unreife myelocyte, [e] promeylocyte S with specific granules, [f] N. myelocyte, [g] metamyelocyte, [h] young granulocyte, [i] jung kernige, [j] young forms, neutrophil, [k] band, staff, stab forms, [l] nonsegmented neutrophils, [m] polymorphonuclear leukocytes, [n] segmented neutrophil leukocytes, [o] large mononuclears, [p] lymphoid cells, [q] plasma cellular reticulum cells, [r] primitive cells, [s] megaloblasts, [t] proerythroblasts, [u] erythroclasts, [v] polychromatic macroblasts, [w] pronormoblasts, [x] normoblasts, [y] macronormoblasts, [z] erythrogones.

[A] Disintegrated cells. [B] 0.03 megakaryocytes; 0.05 histiocytes. [C] 0.2 macrophage; 4.9 lymphoid reticulum cells; 0.01 endothelial cells. [D] 3.1 hematogones; 0.2 megakaryocytes; 0.25 reticulum cells [E] 0.21 hemohistioblast; 0.085 histiocytes; 0.565 megakaryocytes. [F] 0.7 unclassified cells; 0.3 Ferrata cells; 1.6 reticulum cells; 0.1 megakaryocytes. [G] error in recalculating to the basis of 100 per cent nucleated marrow cells. [H] 0.05 megakaryocytes; 2.4 reticulum cells; 1.4 unclassified cells. [I] megakaryocytes. [J] Ferrata, reticulum, and unclassified cells. [K] 7.64 reticuloendothelial cells; 0.72 megakaryocytes; 0.27 Ferrata cells.

ists, pathologists, and clinical pathologists meet, prepare, and publish an approved "Standard Nomenclature for Hematology," clearly defining cell criteria and profusely illustrating the text with numbered colored plates of typical and atypical cells. The terminology and cell criteria used for the headings of the tables in this article are those used, defined, and illustrated in the "Atlas of Hematology" by Osgood and Ashworth (47). However, an internationally approved cell classification and terminology would be heartily endorsed by the authors.

STATISTICALLY UNAVOIDABLE ERRORS IN CELL COUNTING. These errors have been discussed by Dunn (48), Plum (49), Barnett (50), and Segerdahl (30), and a convenient table (51) and chart (52) have been published. For the total nucleated cell count the standard deviation of the dispersion, or sigma, equals the square root of N, which expressed as an equation is $\sigma = \sqrt{N}$, when N is the number of cells enumerated on the counting chamber. For the differential cell count the standard deviation of the dispersion, or sigma, equals the square root of p times q divided by n, which expressed as an equation is $\sigma = \sqrt{\frac{p \cdot q}{n}}$ when p is the number of a specific cell type counted, q is the number of other cells counted, or $n - p$, and n is the total number of cells counted. These errors depend on the laws of chance, are totally independent of all other sources of error in the technic, and are absolutely unavoidable. They can be computed only if the number of cells counted is stated; hence the importance of stating the number of cells counted in any article presenting data obtained by cell counting technics is obvious. With either total cell counts or differential cell counts the standard deviation is proportional to the square root of the number counted; in other words, to double the accuracy quadruple the number of cells counted. Knowing the standard deviation one can determine from tables given in books on statistics the probability that the count will lie outside of plus or minus any specified number of standard deviations; for example, plus or minus one standard deviation includes 68.3 per cent of the determinations; plus or minus two standard deviations includes 95.4 per cent of determinations; and plus or minus three standard deviations includes 99.73 per cent of determinations. Theoretically, if an infinite number of counts were done the limits would be zero and infinity. If only observed extremes are given these counts depend on only two determinations, and by chance they may be well outside plus or minus two standard deviations. It is, therefore, desirable in articles dealing with such material that the individual results be included or else that standard deviations be calculated. Segerdahl (30) has stated that it is safer to determine the upper limit of normal variability as the mean $+ 4\sigma$, but in dealing with biologic material there are serious objections to this, because such limits will include many pathologic results. The range of plus or minus two standard deviations, including approximately 95 per cent of the results, is probably most satisfactory, but it should be remembered that there is one chance in twenty that normal values will lie above or below this range; hence, values near the extremes of this range should be interpreted with

caution. In differential cell counting, when only a few cells of a particular type are present, as is true for many cell categories in marrow preparations, an extremely large number of cells must be counted to bring the percentage error within reasonable limits. Fortunately, the majority of pathologic marrows show such striking deviations from the normal picture that diagnosis is possible by a general survey of the slide without an actual differential cell count. When an accurate differential cell count is necessary at least 500 cells should be enumerated, and if the cell type apparently altered has a percentage of less than 5 it is desirable that at least 2,000 cells be counted, segregating only this cell type and the total number of cells counted, as this will greatly increase accuracy with much less labor than doing a complete differential cell count on 2,000 cells. No differential cell count can be considered accurate unless at least 20 examples of the specific cell type under consideration have been counted. For this reason the reported percentages of megalokaryocytes and some other infrequently occurring cells have been omitted from the tables.

OTHER SOURCES OF ERROR. Aside from the usual sources of error of differential cell counting which have been discussed elsewhere (47), on marrow preparations there may be errors from failure to shake the specimen adequately so that all cells are suspended. Variations in different punctures made at the same time from adjacent areas are introduced by withdrawing a sample from a nonhomogeneous tissue and are especially great if a small sample is taken, and may be huge if the hemocytometer pipette is filled directly from a fragment of marrow. The uneven distribution of cells of various size on smears may also be a cause of variation (53). Another important source of error is failure to include disintegrated cells in the differential cell count; since these cells are included in the total nucleated cell count it would seem necessary to include them in the differential cell count if the correct proportions of other cell types is to be obtained. Furthermore, it has been found to be of great diagnostic value to include them, since an increase in disintegrating cells is one of the most constant and important criteria for the diagnosis of leukemia. As yet unpublished data obtained by comparison of supravital preparations on blood, marrow, and marrow cultures with Wright's stained smears of the same material indicated a high correlation between the number of nonmotile cells with stained nuclei in the supravital preparations and disintegrated cells in the stained preparations, suggesting that disintegrated cells in well made stained preparations represent dead and dying cells and not merely mechanically produced artefacts (54).

TOTAL NUCLEATED CELL COUNTS. In table 1 the data available on total nucleated cell counts in adults are summarized. Data of Wiener (55), Vogel and Bassen (56) and Gordon (57) are not included, because they failed to include the number of subjects examined. Napier and Gupta, and Pitts and Packham took 2 cc., all others took 0.2 cc. or less of marrow, except Tempka and Braun and De Renzi and Fuortes who failed to state how much marrow was aspirated. All of the authors whose data is included in table 1 stated that their subjects were healthy or gave further evidence that this was true. The

data of Greif (22, 29) were not included, since his counts were made on 0.1 cc. of marrow aspirated and placed in a watch glass in which filter paper was dipped to "absorb the blood and tissue fluid," and the counts then made on the remainder, and because only the extremes which would be based on 2 rather than 104 cases were given. There was so much variability in means and ranges that it did not seem justifiable to calculate a weighted mean for this series. The variability is probably due to marked variations in the technic, as previously discussed. Segerdahl's subjects were presumably Swedish, and she segregated them into age and sex groups. There was apparently no significant difference between the sexes or between the younger subjects and the old age group; so for the purposes of the table we have calculated the weighted means for the entire adult series. The subjects of Napier and Gupta were residents of India. Those of De Renzi and Fuortes were Italians between 15 and 43 years of age, mostly between 20 and 25. The subjects examined by Pitts and Packham were mostly young Canadian nurses. Pitts and Packham also report data on 40 pregnant women, finding an average total nucleated cell count of 36,760, with a range of 14,400 to 125,000. Apparently a slightly but significantly higher count occurs during pregnancy. Gormsen's subjects were Danish, ranging in age from 15 to 72 years. The data on total nucleated cell counts in children are given in table 2. While the number of subjects is too small and the ages too variable to justify averaging the results it is apparent that higher counts are found in newborn infants and young children than in the older age groups. All of the authors aspirated 0.1 to 0.2 cc. of marrow, yet it is readily possible to obtain 1 cc. or more in children of these age groups, avoiding the error inherent in using such a small quantity of material.

DIFFERENTIAL CELL COUNTS ON STERNAL MARROW FROM HEALTHY INDIVIDUALS. The data available on differential cell counts on aspirated sternal marrow from healthy individuals are summarized in table 3. Many articles (41) were excluded from the tables because insufficient data were given to justify comparison with those used, and several articles (58) which were of value in showing the normal ranges did not give their means and were, therefore, excluded. The actual names for cells used by the individual authors are grouped at the bottom of the table and are referred to by reference letters of the alphabet following the corresponding names. The capital letters in the miscellaneous column refer to the names and percentages of the cells included in that column. These are given below the table. Since it is well recognized that the various blast cells are very difficult (if not impossible) to differentiate from each other it is undoubtedly probable that the granuloblast (myeloblast) column includes small numbers of lymphoblasts and monoblasts as well as, with some authors, karyoblasts (megaloblasts). Many authors failed to differentiate progranulocytes (promyelocytes) from granulocytes (myelocytes), and very few authors separated progranulocytes S (promyelocytes I) from the neutrophil granulocyte (myelocyte). This has been indicated by brackets. It is probable also that some of the cells in the column headed neutrophil metagranulocytes (metamyelocytes) would have been placed in the column headed neutrophil granulocytes (myelo-

cytes) by other authors and in the rhabdocyte (staff cell) column by still others. There was also a wide difference in the line of separation between the neutrophil rhabdocytes (staff cells) and neutrophil lobocytes (polymorphonuclear neutrophil leukocytes). Some authors divided them on the basis of whether they were filamented or nonfilamented, which we recommend, and others segregated the lobocytes (polymorphonuclear neutrophil leukocytes) from the rhabdocytes (staff cells) when there was a slight indentation of the nucleus. Because of the relatively small numbers it did not seem practical to include the different stages of development of the eosinophils or of the basophils separately. Notwithstanding the grouping of all forms of eosinophils together there is a surprising variation in results. If any cell should be easily recognized and identified as the same by all investigators they would certainly seem to be the eosinophils. The high mean value of 9.1 per cent eosinophils was in Indian subjects, which makes one suspect that parasitic infestation may have been present.

Criteria for classification of nucleated erythrocytes were even more variable and difficult to interpret than those given for the leukocytes. This difficulty has been discussed elsewhere (59), so it will not be repeated here, except to emphasize that different authors (46) use the term "megaloblast" with entirely different meanings which are mutually exclusive.

The miscellaneous column includes a number of different types of cells that could not justifiably be placed under any other heading. The names used by the original authors for these cells will be found beneath the table with the corresponding capital letter. Young and Osgood, and Pitts and Packham included disintegrated cells in the differential cell count, and allowance must be made for the resulting reduction in the percentages of the other cell types. Megalokaryocytes are too few in number to be counted accurately in the ordinary differential cell count. Limarzi and Schleicher (60), by another method, have estimated the number of these cells as 58.8 per million nucleated bone marrow cells, or approximately 0.006 per cent. Because Morrison and Samwick, in their original article, gave percentages for the granulocytic series and the nucleated erythrocytic series, totalling 100 per cent for each series it was necessary to recalculate their data from the ratios of the granulocytic series to the nucleated erythrocytic series, or the granulocytic series to the lymphocytic series, and of the granulocytic series to the plasmacytic series which were given, and the 3.0 per cent in the miscellaneous column equals the error in recalculation to a basis of a differential cell count of all nucleated cells equal to 100 per cent. The authors agree with Segerdahl (30) and Naegeli (61) that the category of Ferrata cells should probably be discarded, since they apparently represent disintegrated granulocytes (myelocytes), progranulocytes (promyelocytes), and granuloblasts (myeloblasts), and occasionally disintegrating promonocytes from the descriptions and illustrations given (62).

Although the series is obviously not homogeneous enough in criteria of cell identification to permit accurate interpretation of the means, it did seem worth while to calculate the weighted means of the cell groups. In making these calculations it was necessary to group together certain categories because of

much overlapping. The range of means is also included to give an idea of the wide differences obtained by different authors. This seems to be due largely to differences in cell identification. The weighted means of the total granulocytic series, the lymphocytic series, the monocytic series, and the total nucleated erythrocytic series were also calculated so that the ratios could be computed. The ratio for the entire series of cases in table 2 of granulocytic series:nucleated erythrocytic series was 63.0:17.21, or 3.67:1.0; of the granulocytic series: lymphocytic series was 63.1:14.2, or 4.44:1.0; of the granulocytic series:monocytic series was 63.21:1.71, or 37:1.

Segerdahl (30) was the only author who studied men and women of comparable ages, and she found no significant differences in the values for the granulocytic series and very slightly lower values for the nucleated erythrocytic series in women. Some idea of the similarities of results in the two sexes in similar age groups can be obtained by comparing the data of Young and Osgood with those of Pitts and Packham. These studies were made by the same technic with identical criteria for cell identification. The former studied only males, and the latter studied only females.

The data available on differential cell counts in infants and children are tabulated in a similar manner in table 4. The tabulation was made in order of increasing age from premature infants to children up to 15 years of age. The averages were not computed, since age groups obviously differed greatly. From a comparison of Tables 3 and 4 it is apparent that the differences in differential cell counts in children and adults are relatively slight compared to the variations in the figures of different investigators on the same age group. If there is any significant difference it is a slightly higher percentage of granuloblasts (myeloblasts) and progranulocytes (promyelocytes) and a slightly lower percentage of neutrophil lobocytes (polymorphonuclear neutrophil leukocytes) in children.

COMMENT. It was hoped, when this review was undertaken, that from a compilation of the data available specific recommendations could be made as to normal standards for aspirated marrow. It is apparent from the data presented that this must await further studies and a more general agreement on the technic of marrow aspiration and the handling of the material after aspiration, and especially on the acceptance of more uniform criteria of cell identification and nomenclature.

Until such time as further data are available it is suggested that investigators use the figures in table 5 as tentative normal standards. The figures for the larger amounts aspirated were calculated from the data of Napier and Gupta (63) and Pitts and Packham (64) for total nucleated cell counts, and from the data of Young and Osgood (13) and Pitts and Packham (64) for differential cell counts. These figures should be used if 0.5 to 10 cc. of marrow is aspirated and disintegrated cells are included in the differential cell count, as is recommended. The figures for the smaller amounts aspirated were calculated from the data of Segerdahl (30), and should be used if the investigator uses her technic and criteria of cell identification, and prefers to omit disintegrated cells and to aspirate 0.2 cc. or less of marrow, notwithstanding the disadvantages which

TABLE 4

Differential cell counts reported on sternal marrow of healthy infants and children

AUTHOR	NO. OF CASES	AGE	GRANULOBLASTS	PROGRANULOCYTES		GRANULOCYTES	METAGRANULOCYTES	RHABDOCYTES	LOBOCYTES	EOSINOPHILS	BASOPHILS	LYMPHOCYTES	MONOCYTES	NUCLEATED RBC	MISCELLANEOUS
				A	S										
Lichtenstein and Nordenson	17	Premature	3.98[a]	4.18[b]	(	14.36[c])	7.67[d]	4.26[f]	15.51[g]	2.4	0.0	13.8	1.97	16.92	5.76[A]
(16)	8	Newborn	4.0[a]	3.66[b]	(	12.5[c]	16.09[d]	7.34[f]	17.72[g]	2.22	0.0	15.53	4.12	14.24	1.18[B]
Shapiro and Bassen (66)	35	Newborn–24 hrs.	0.8[a]	(		16.3[c])	(33.9[h]	)	7.0[g]	2.6	0.0	3.8	0.0	31.9	3.9[C]
		7 days	1.5[a]	(		19.7[c])	(43.5[h]	)	10.5[g]	2.3	0.0	6.2	0.0	11.6	5.0[D]
Veeneklaas (23)	3	Under 14 days	2.0[a]	2.4[b]	(	13.2[c])	8.2[d]	24.1[f]	12.9[g]	4.0	0.0	19.0[i]	0.0	12.0	2.8[E]
Vogel and Bassen (56)	12	Under 24 days	1.94[a]	0.21[b]	(	21.86[c]	24.55[h]	)	13.6[g]	2.51	0.0	10.5	0.0	19.75	4.95[F]
Kato (73)	18	1–12 months	2.0[j]	3.1[k]	7.2[l]	9.4[c]	11.8[d]	12.6[f]	4.5[g]	5.6	0.0	17.4	0.5	26.0	0.0
Veeneklaas (23)	65	14 days to 3 yrs.	2.5[a]	2.4[b]	(	9.8[c])	10.0[d]	10.4[f]	8.6[g]	1.35	0.0	36.7	0.6	13.3	1.6[G]
Diwany (24)	5	15 mos. to 2 yrs.	1.6[a]	0.85[b]	(	17.0[c])	16.6[d]	6.4[f]	7.0[g]	4.8	0.0	27.7	0.05	19.4	0.0
Kato (73)	32	1–15 years	2.1[j]	3.1[k]	5.6[l]	10.3[c]	10.9[d]	17.3[f]	6.5[g]	5.6	0.4	17.3	1.6	19.3	0.0
Jacobsen (43)	13	2–9 years	1.26[a]	2.83[b]	(	24.86[c])	21.02[d]	4.46[f]	8.3[g]	4.14	0.19	18.22	0.98	12.47	1.26[H]
Vogel and Bassen (56)	29	2–12 years	1.93[a]	0.49[b]	(	17.1[c])	(24.79[h]	)	15.16[g]	3.95	0.06	7.9	0.0	23.04	5.15[I]
Veeneklaas (23)	33	over 3 yrs.	1.5[a]	2.2[b]	(	9.4[c])	11.5[d]	13.2[f]	14.4[g]	1.6	0.0	26.0	0.6	14.0	2.2[J]
Diwany (24)	10	3½–5 yrs. 5 mos.	0.88[a]	0.48[b]	(	17.1[c])	20.3[d]	12.0[f]	10.5[g]	6.67	0.0	19.9	0.4	11.5	0.0

[a]myeloblast; [b]promyelocyte with azur granules; [c]N. myelocyte; [d]N. metamyelocyte; [f]rod or staff forms, N.; [g]N. segmented neutrophils; [h]N. non-segmented neutrophils; [i]lymphoblasts and lymphocytes; [j]myeloblasts (Totipotent stem cells); [k]myeloblastic leukoblast ;[l]promyelocyte with specific granules.

[A]0.53 unclassified; 0.59 reticulum cells; 0.06 megakaryocytes. [B]0.59 unclassified; 4.58 reticulum cells; 0.24 plasma cells; 0.35 megakaryocytes. [C]3.8 hematagones; 0.1 megakaryocytes. [D]0.1 megakaryocyte; 4.8 hematagone; 0.1 reticulum cells. [E]0.2 megakaryocyte; 4.65 hematagones; 0.1 reticulum cells. [F]0.1 megakaryocytes; 5.0 hematagones; 0.05 reticulum cells. [G]0.3 megakaryocytes; 0.8 reticulum cells; 0.2 plasma cells; 1.5 Ferrata cells. [H]0.3 megakaryocytes; 0.5 reticulum cells; 0.5 plasma cells; 0.3 Ferrata cells. [I]0.4 megakaryocytes; 0.8 reticulum cells; 0.6 plasma cells; 0.4 Ferrata cells. [J]0.9 reticulum cells; 0.13 plasma cells; 0.05 Ferrata cells.

have been pointed out. The weighted means have been smoothed and the 95 per cent ranges so calculated as to include approximately plus or minus two standard deviations. The data on differential cell counts of Napier and Gupta (63) were not used in the calculation of the differential cell counts because of the omission of disintegrated cells and of their high eosinophil values which are probably more frequently encountered in residents of India than in residents of Europe and the western hemisphere.

TABLE 5

Tentative recommended standards

	0.5 TO 10.0 CC. ASPIRATED*		0.05 TO 0.5 CC. ASPIRATED†		
	Weighted means	95% Range	Weighted Means	95% Range	
Total nucleated cell count	35,000	10,000–100,000	75,000	10,000–160,000	
Granuloblasts	0.40	0.0– 1.0	1.25	0.0– 2.4	Myeloblasts
Progranulocytes A.	1.4	0.0– 3.0	1.50	0.0– 3.0	Promyelocytes
Progranulocytes S.	1.0	0.0– 3.0			
Granulocytes	3.2	0.0– 9.0	15.0	8.0–22.0	N. myelocytes
Metagranulocytes	6.5	3.0–10.0	15.5	8.0–23.0	N. metamyelocytes
Rhabdocytes	24.0	17.0–33.0	9.0	6.0–12.0	N. staff cells
Lobocytes	15.0	5.0–25.0	22.0	15.0–28.0	Polymorphonuclears
Eosinophils	2.0	0.0– 4.0	3.0	1.0– 5.0	Eosinophils
Basophils	0.2	0.0– 0.5	0.2	0.0– 0.4	Basophils
Lymphocytes	14.0	3.0–25.0	18.5	7.0–30.0	Lymphocytes
Monocytes	2.0	0.0– 4.0	2.0	0.0– 4.0	Monocytes
Karyoblasts	0.2	0.0– 1.0			
Prokaryocytes	2.0	0.0– 4.0			
			12.0	3.0–21.0	Erythroblasts
Karyocytes	6.0	4.0– 8.0			
Metakaryocytes	3.0	1.0– 5.0			
Disintegrated cells	19.0	10.0–30.0			

* The smoothed weighted mean and the 95 per cent range of the total nucleated cell count were calculated from the data of Napier and Gupta (63) and Pitts and Packham (64), and those of differential cell counts were calculated from the data of Young and Osgood (13) and Pitts and Packham (64).

† The smoothed weighed means and 95 per cent ranges of the total nucleated cell count and the differential cell counts were calculated from the data of Segerdahl (30).

Unquestionably the most important suggestions that are justified on the basis of this review are that further studies of the cytologic composition of marrow obtained by sternal aspiration are urgently needed, as well as further studies on necropsy material obtained within a few minutes after death. It is suggested that when such studies are made the following conditions should be fulfilled and the data included in the article: adequate evidence of the health of the subject or, if necropsy material is used, of the health of the subject prior to death; the age, sex, and race of the subjects; the geographic location and the

altitude at the locale of the study; the number of subjects studied; the site and technic of the puncture; the amount of material aspirated and its preparation for study; the total nucleated cell count on a thoroughly mixed specimen with the number of cells actually counted; the methods used in making and staining smears; differential cell counts which must be done on the thin areas of the smears where the erythrocytes do not touch each other to avoid errors in identification; the total number of cells counted in the differential cell count; and, of especial importance, the criteria of cell identification, preferably with a reference to some readily available atlas in which all types of cells are illustrated, at least until some international standard of cell identification and nomenclature is agreed upon. It is far easier to evaluate results if the actual figures on each subject as well as the averages and statistical constants are given.

The true normal anatomy of human marrow will not be known until comparative studies by all the methods available, as outlined in the body of this article, are made on fresh necropsy material from the bodies of previously healthy persons who have died from sudden traumatic causes.

Notwithstanding the limitations which have been pointed out and the lack, as yet, of accurate normal standards, the widespread use of sternal puncture is a testimonial to its value in the differential diagnosis of aleukemic and subleukemic leukemias, aplastic anemia, other puzzling anemias, malaria, leishmaniasis, multiple myeloma, and the lipoid histiocytoses, and to the value of bacteriologic cultures of marrow in subacute bacterial endocarditis and other bacteriemias.

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CHEMICAL METHODS FOR THE DETERMINATION OF DEATH BY DROWNING

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If drowning be defined as suffocation caused by the inhalation of fluid, the potential diagnostic significance of two categories of postmortem chemical findings should be considered. One includes the changes that might be expected to result from obstructive asphyxia. The other includes the changes that might be expected to result from an exchange of water or electrolytes between the inhaled fluid and the blood of the pulmonary capillaries.

It is obvious that the evidence derived from chemical investigation of postmortem material will be of limited value in determining whether or not death has resulted from obstructive asphyxia. Most of the chemical alterations that would constitute evidence of asphyxia if recognized during life are likely to be simulated by agonal or masked by postmortem change.

Chemical evidence that asphyxia is the primary cause of death in drowning was first demonstrated experimentally by Brouardel whose findings were subsequently confirmed and extended by Lougheed, Janes and Hall. It was observed by the latter that the oxygen saturation of arterial blood drops to approximately 15 volumes per cent during the first few minutes after submersion but is reduced to between 2 and 3 volumes per cent by the end of 6 minutes and is almost completely exhausted within 7 or 8 minutes. They observed that the carbon dioxide content of whole blood rises rapidly during the first few minutes of submersion but by the end of 4 minutes drops below the pre-experimental level. Subsequently it undergoes a slowly progressive decline. A similar antemortem drop was observed in the total carbon dioxide of the blood of animals killed by tracheal compression.

If depletion of oxygen and retention of carbon dioxide are the primary effects of obstructive asphyxia a redistribution of electrolytes between cells and plasma and a lowering of the pH of the blood may be characterized as secondary attributes. Although these secondary changes might be of diagnostic value if recognized during life their presence in postmortem blood can have no meaning beyond the fact that circulation has ceased, and that death has occurred. Other and, from a diagnostic standpoint, equally unimportant asphyxial changes in the composition of the blood result from hemoconcentration (Collip; Peters and Van Slyke; Inouye and Uchimura).

Despite the fact that the absence of oxygen in a postmortem sample of blood has no diagnostic significance its presence may provide conclusive evidence that death did not result from asphyxia. Goggio found that the oxygen content of arterial blood is regularly reduced to a trace after asphyxial death whereas in animals dead of sudden cardiac arrest (electrical shock) many hours elapse before the residual oxygen of arterial blood is reduced to a level consistent with death from asphyxia.

That asphyxia commonly results in a marked rise in the glucose concentration of the blood has been known since the early investigations of Claude Bernard. Although various stimuli may cause a sudden outpouring of adrenalin resulting in hepatic glycogenolysis and hyperglycemia, Hill has recently stressed the fact that a high glucose concentration in an appropriate sample of blood may provide evidence of confirmatory value in the recognition of acute asphyxial death. It should be remembered, however, that the finding of a high glucose concentration in a postmortem sample of blood from the right side of the heart does not necessarily indicate an antemortem state of hyperglycemia, nor does the absence of glucose from a postmortem sample of blood indicate hypoglycemia during life. A sharp, evanescent elevation of the glucose in the inferior vena cava and right side of the heart as a result of hepatic glycogenolysis is a normal postmortem phenomenon. If a postmortem sample of blood is to be taken for glucose determination it should be from the left heart and it should be taken as soon after death as possible to avoid the artefact of postmortem glycolysis.

Chemical changes due to the exchange of water and electrolytes between inhaled fluid and the blood in the pulmonary capillaries. More than a century ago Mayer demonstrated that the lungs are capable of absorbing water and certain water solutes. That the absorbed material may alter the composition of the blood was indicated by the observation that within two minutes after the fluid had reached the pulmonary alveoli certain chemicals present in it could be demonstrated in the chamber of the left ventricle.

Peiper reported that a dog could inhale as much as 250 cc. of inert fluid in the course of an hour without suffering from dyspnea. Sehrwald described an experiment in which 775 grams of fluid were introduced into the lungs of a 16 lb. dog over a period of 2 hours without causing undue distress. Clinical evidence indicated that the fluid was absorbed by the end of 5 days. Colin introduced 25 liters of water into the trachea of a horse over a period of 6 hours without significant interference with pulmonary ventilation. The horse was protected against hydremia, however, by the withdrawal of 6 kilograms of blood during the course of the experiment. Karpovich in an experimental study with rats tied the esophagus of his animals before submerging them and observed that the amount of fluid retained in their bodies after drowning often exceeded the weight of the lungs by more than 100 per cent.

The question of whether or not submersion of a body after death may result in changes in the composition of the blood similar to those caused by drowning has been studied by various investigators. Small amounts of water may enter the trachea and bronchi of the bodies of men (Müller) and of animals (Wachholz) that have been submerged after death from causes other than drowning. It was found, however, that water does not enter the small air passages or pulmonary alveoli in such circumstances. That diffusion between the water that has entered the air passages after death and the blood in the pulmonary capillaries is not sufficient to produce significant changes in heart's blood was shown by Jetter and Moritz.

Various investigators have added chemical tracers to the fluid in which ani-

mals were drowned in order to demonstrate absorption from the lungs. Doehne in 1857, Falk in 1869, and Sehrwald in 1886 drowned animals in fluid containing potassium ferrocyanide and subsequently demonstrated the presence of that chemical in the blood stream. More recently Lochte and Danziger added potassium iodide and Lochte added calcium oxide to the fluid in which animals were drowned. Winternitz demonstrated the absorptive capacity of the respiratory membrane by adding phenolsulphonphthalein to water and observed that dye is absorbed as rapidly from the alveoli as it is when injected intramuscularly.

Brouardel conducted the first experimental investigation of the mechanism of death in drowning by what might be characterized as modern physiological methods. Stimulated in part by his own observations on the absorption of inhaled water and in part by his appreciation of the unreliability of postmortem pathological changes in making a diagnosis of death by drowning, he undertook to investigate the diagnostic significance of disproportionate intracardiac hemodilution. From observations made in collaboration first with Vibert and later with Loye he concluded that a comparison of the hemoglobin and erythrocyte content of right and left heart's blood constituted a practical method of recognizing death by drowning. By both procedures it was observed that disproportionate dilution of left heart's blood occurred after drowning in fresh water. Brouardel's observations were subsequently confirmed by Paltauf.

When consideration is given to the nature of the terminal circulatory failure in death by drowning it is not surprising that at autopsy the blood in the left side of the heart may differ from that in the right. After consciousness has been lost and the glottic spasm relaxed, enough water is usually inhaled to change the composition of the blood passing through the pulmonary capillaries. It can be assumed that the nature and degree of change will depend on the differences that exist between the osmotic pressure of the intra-alveolar fluid and that of capillary blood and on the length of time that circulation is maintained during and after the inhalation of water. If heart failure in drowning were sudden so as to cause an abrupt and complete cessation of circulation the finding of significant postmortem differences in the composition of the blood in the two sides of the heart would be unlikely. It has been observed however that in experimental animals there is electrocardiographic evidence of feeble contractions for as long as 30 to 40 minutes after all respiratory efforts have ceased and after the pulse has become imperceptible (Lougheed, Janes and Hall). During this period of agonal circulatory failure it is to be expected that blood which has been altered in passing through the pulmonary capillaries will be gradually pooled in the dilated vessels of the systemic circulation so that each feeble ventricular discharge will increase the change in the left heart's blood without contributing correspondingly to the change in the right side. Thus, when drowning has occurred in fresh water it would be reasonable to expect to find disproportionate dilution of left heart's blood. By the same reasoning it might be expected that when drowning occurs in an aqueous medium containing a sufficient concentration of electrolytes to raise its osmotic pressure above that of blood, a disproportionate increase in these electrolytes will be found in a postmortem

sample of left ventricular blood. Sea water which usually has a mineral concentration more than three times greater than that of the blood represents such a medium.

Presumptive evidence of disproportionate dilution might be obtained from a comparison of the hemoglobin, the iron, or the number of red cells in samples taken from the right and left sides of the heart. It is obvious, however, that the significance of such observations will depend on how truly the postmortem samples represent the plasma-cell ratio of the blood at the moment of death. Postmortem settling of erythrocytes or movement of plasma can and does account for uncontrollable factors of error.

Two other types of criteria for judging the occurrence of agonal diffusion may be considered. One is by the recognition of changes in the molecular concentration of blood samples by such physical characteristics as specific gravity, freezing point, or electrical conductivity. The other is the identification by chemical means of alterations in the electrolyte concentration of the blood. Thus, after drowning in fresh water a reduction might be expected in the specific gravity, the Δ of the freezing point and the electrical conductivity of the left heart's blood, whereas after drowning in sea water the reverse should be true. After drowning in fresh water electrolytes in the left ventricle might be expected to be disproportionately diminished whereas an increase would be expected after drowning in sea water.

Carrara was among the first to apply physical methods to investigate the effects of drowning on the molecular concentration of heart's blood. As a result of determinations of specific gravity, freezing point and electrical conductivity he concluded that left ventricular blood is disproportionately diluted after drowning in fresh water and disproportionately concentrated after drowning in sea water. He also observed that postmortem alterations in blood independently of drowning may account for equally great changes and concluded, perhaps prematurely, that it is impossible in non-experimental conditions to make a categorical statement regarding the diagnostic significance of changes found in postmortem samples.

During the succeeding 25 years the diagnostic significance of hemo-dilution and -concentration as disclosed by a variety of physical tests was the subject of conflicting opinions. Placzek recommended the diagnostic value of determining the specific gravity of right and left heart's blood. Revenstorf proposed cryoscopic examination and although he recognized the fact that postmortem changes, independent of drowning, often result in pronounced and unpredictable alteration in the density of the blood, concluded that the procedure was frequently of diagnostic value in borderline cases. He called attention to the fact that postmortem depression of the freezing point normally occurs more rapidly in blood than it does in spinal fluid, whereas, after death by drowning in fresh water, the freezing point of left ventricular blood was likely to be higher than that of the spinal fluid. Balthazard concluded that cryoscopic examination was superior to determining either the hemoglobin or erythrocyte content of the blood, whereas Wachholz and Horosziewicz doubted the diagnostic reliability of either

procedure. Canuto on a basis of uncontrolled data concluded that recognition of disproportionate changes in the refractive index of right and left heart's blood provides a basis for establishing a postmortem diagnosis of death by drowning in man. Yamakami, and later Inouye and Uchimura, confirmed the already established fact that changes in the freezing point and refractive index occur in the blood of experimentally drowned animals. Schwarzacher, in a reinvestigation of the significance of alterations in electrical conductivity of the blood of persons dead of drowning and of persons dead of causes other than drowning, concluded that the postmortem artefact rendered such tests of little or no practical value.

Although Stockis was first to report differences in the chloride content of right and left heart's blood after death by drowning he was not particularly impressed with their diagnostic significance and not until 1921 when Gettler reported a relatively large series of determinations on human subjects was their potential diagnostic significance appreciated. On a basis of chloride determinations of right and left heart's blood of 22 individuals dead of causes other than drowning, 19 deaths by drowning in salt water and 3 deaths by drowning in fresh water, Gettler concluded that a difference of 25 mgm. NaCl establishes death by drowning. He called attention to the fact that a significant difference might fail to develop if death resulted from the shock of immersion rather than drowning or if there were a patent foramen ovale. According to Gettler a high chloride value in the left ventricle indicates death by drowning in salt water and low chloride value signifies drowning in fresh water. It was observed that the longer the interval between inhalation of water and death the greater the differences were likely to be.

Since the publication of Gettler's observations the significance of the chloride content of the blood in relation to death by drowning has been the subject of numerous investigations and considerable diversity of opinion. In order to evaluate the published data in their entirety all chloride values have been converted to milli-equivalents per liter and arranged in tabular form. Some authors described analytical procedures and others did not. From the available descriptions it is apparent that all methods were not of equal sensitivity and certain differences in the data are probably attributable to this fact.

Blood chlorides (rabbits) after death from causes other than drowning. It is obvious that knowledge of the nature and extent to which the blood chlorides may be altered incident to death from causes other than inhalation of water is pre-requisite to the evaluation of changes regarded as pathognomonic of drowning. The chloride concentrations of whole blood or serum of 15 rabbits taken before and immediately after death by strangulation are shown in table 1. Antemortem samples were from the ear vein and postmortem samples from the right side of the heart. In every instance the postmortem was higher than the antemortem value, the increases ranging from 3 to 9 mEq/L in whole blood and from 2 to 8 in serum. So far as can be judged from the limited number of observations the increases in serum and whole blood are of approximately the same order of magnitude. In the 4 animals reported by Inouye and Uchimura it was observed that the refractive index of the serum was increased and that its freezing point was reduced.

In another series of control experiments (see table 2) the chloride concentration of postmortem samples of right and left heart's blood taken immediately after death by strangulation was determined. It appears that the increased concentration observed after death from mechanical asphyxia (strangulation) does not affect the blood in one side of the heart more than it does in the other. The recorded differences are not regarded to be significant.

TABLE 1

Chloride content of rabbits' blood before and immediately after death by tracheal compression (milli-equivalents per liter)*

ANTE-MORTEM	POST-MORTEM	DIFFERENCE, PM–AM	REPORTED BY	ANTE-MORTEM	POST-MORTEM	DIFFERENCE, PM–AM	REPORTED BY
91	99	8	Yamakami	87	96	9	Yamakami
85	90	5	Yamakami	87	94	7	Yamakami
85	95	10	Yamakami	83*	85*	2	Inouye and Uchimura
89	101	12	Yamakami	100*	106*	6	Inouye and Uchimura
90	93	3	Yamakami	97*	105*	8	Inouye and Uchimura
87	91	4	Yamakami	92*	97*	5	Inouye and Uchimura
88	91	3	Yamakami				

* Values designated by * represent serum. All other values are for whole blood.

TABLE 2

Chloride content of right and left heart's blood of rabbits immediately after death by tracheal compression (milli-equivalents per liter)*

RIGHT HEART	LEFT HEART	DIFFERENCE, LH–RH	REPORTED BY	RIGHT HEART	LEFT HEART	DIFFERENCE, LH–RH	REPORTED BY
97	97	0	Yamakami	96	94	−2	Yamakami
93	92	−1	Yamakami	93	93	0	Yamakami
85	85	0	Yamakami	98	97	−1	Yamakami
91	91	0	Yamakami	98	98	0	Yamakami
101	101	0	Yamakami	99	100	1	Yamakami
93	93	0	Yamakami	94	95	1	Yamakami
91	92	1	Yamakami	106*	106*	0	Inouye and Uchimura
91	91	0	Yamakami	106*	107*	1	Inouye and Uchimura
91	90	−1	Yamakami	111*	112*	1	Inouye and Uchimura
93	91	−2	Yamakami				

* Values designated * represent serum. All other values are for whole blood.

Blood chlorides (rabbits) after death by drowning in fresh water. The chloride concentrations of right and left heart's blood of 20 rabbits taken immediately after death by drowning in fresh water are shown in table 3. It is apparent that in every animal there has been a significant and disproportionate reduction in the chloride content of the blood in the left side of the heart which persisted after death. The mean value of the differences between the 2 sides of the heart was 17 mEq/L with a minimum of 6 and a maximum of 27. Although the data shown in table 3 do not indicate whether or not the right heart's blood was also diluted,

the pre-experimental blood chloride values for the 2 animals reported by Inouye and Uchimura did not indicate that such was the case. The pre-experimental serum chloride values for venous blood from these 2 animals were respectively 101 and 107 mEq/L.

Blood chlorides (rabbits) after drowning in salt water. The chloride concentrations of right and left heart's blood of 15 rabbits observed immediately after death

TABLE 3

Chloride content of right and left heart's blood of rabbits immediately after death by drowning in fresh water (milli-equivalents per liter)*

RIGHT HEART	LEFT HEART	DIFFERENCE, LH-RH	REPORTED BY	RIGHT HEART	LEFT HEART	DIFFERENCE, LH-RH	REPORTED BY
104*	80*	−24*	Inouye and Uchimura	85	71	−14	Yamakami
105*	78*	−27*	Inouye and Uchimura	73	55	−18	Yamakami
79	71	−8	Yamakami	82	67	−15	Yamakami
80	64	−16	Yamakami	83	60	−23	Yamakami
85	63	−22	Yamakami	80	58	−22	Yamakami
94	88	−6	Yamakami	74	62	−12	Yamakami
79	66	−13	Yamakami	80	70	−10	Yamakami
88	62	−26	Yamakami	85	69	−16	Yamakami
82	65	−17	Yamakami	88	72	−16	Yamakami

* Values designated * represent serum. All other values are for whole blood.

TABLE 4

Chloride content of right and left heart's blood of rabbits immediately after drowning in salt water (milli-equivalents per liter)*

RIGHT HEART	LEFT HEART	DIFFERENCE, LH-RH	REPORTED BY	RIGHT HEART	LEFT HEART	DIFFERENCE, LH-RH	REPORTED BY
135*	148*	13	Inouye and Uchimura	112	151	39	Yamakami
116*	123*	7	Inouye and Uchimura	102	114	12	Yamakami
120*	137*	17	Inouye and Uchimura	117	139	22	Yamakami
129*	154*	25	Inouye and Uchimura	116	136	20	Yamakami
106	131	25	Yamakami	125	141	16	Yamakami
137	166	29	Yamakami	117	132	15	Yamakami
134	169	25	Yamakami	120	146	26	Yamakami
127	169	42	Yamakami				

* Values designated * represent serum. All other values are for whole blood.

from drowning in salt water are shown in table 4. In every instance the concentration was greater in the left than in the right side of the heart. The mean value of the differences for whole blood (11 pairs of samples) was 25 mEq/L and for serum (4 pairs of samples) was 16.

It is apparent not only that the chloride concentrations in the left heart were higher than those in the right side of the heart but also that the chloride concentrations on both sides are higher than would be expected in animals dead of causes

other than drowning. The difference between chambers ranged from 7 and 42 mEq/L. In addition to the data shown in table 4 antemortem chloride values were available for the 4 rabbits reported by Inouye and Uchimura. In each of these the chloride content of the postmortem samples of venous blood (right heart) was significantly higher than that of the corresponding antemortem samples. The differences between the antemortem and postmortem samples in the 4 animals were respectively 13, 7, 17 and 25 mEq/L.

TABLE 5

Chloride content of blood serum taken from dogs before and at varying intervals after death by tracheal compression (Jetter and Moritz) (milli-equivalents per liter)

ANTEMORTEM	POSTMORTEM INTERVAL	RIGHT HEART	LEFT HEART	DIFF. BETWEEN A.M. AND P.M. SAMPLES OF VENOUS BLOOD, P.M.–A.M.	POSTMORTEM DIFF. BETWEEN RH AND LH, LH–RH
	hrs.				
115	¼	117	121	2	4
	6	114	119	−1	5
	24	78	78	−37	0
	48	74	72	−41	−2
116	¼	117	121	1	4
	12	88	100	−28	12
	24	78	86	−38	8
114	6	114	116	0	2
	12	107		−7	
	24	84	95	−30	11
	48	65		−49	
	72	60		−54	
134	24	72	78	−62	6
	48	71	68	−63	−3
	72	65	64	−69	−1
106	24	79	89	−27	10
	72	77	77	−29	0

From determinations of chlorides, freezing point, and refractive index of rabbit serum before and after death Inouye and Uchimura concluded that whereas the reduction of chlorides incident to drowning in fresh water is consistent with simple dilution by diffusion, the chloride concentration after salt water drowning could not be accounted for by diffusion alone.

Blood chlorides (dogs) after death from causes other than drowning. The chloride content of blood serum taken from dogs before and at different intervals after death by tracheal compression is shown in table 5. In all animals there was a progressive decline in the chloride concentration of both right and left heart's blood as the interval between death and sampling lengthened. Significant

diminution first became apparent between 6 and 12 hours after death. The percentile loss at the end of 24 hours from the right heart of the 5 dogs shown in table 5 was respectively 32, 33, 26, 46 and 25. The corresponding percentile chloride decreases in the left heart were 32, 26, 17, 42 and 16. Thus it appears that in dogs postmortem reduction of serum chlorides tends to take place more rapidly in the right than in the left side of the heart. In the case of the former the antemortem values were reduced by between a quarter and a half during the first 24 hours after death. As a result of variations in the rate of chloride loss from the right and left sides of the heart of the same animal differences as great as 12 mEq/L may be encountered during the first 12 hours after death.

That the postmortem reduction in chlorides occurred more rapidly from serum than from whole blood was apparent from unpublished experimental data of Jetter and Moritz. It was furthermore observed that although the environmental temperature had little or no effect on the rate of serum chloride loss during the first day after death the rapidity of chloride loss during the second and succeeding days was enhanced by putrefaction.

Several factors undoubtedly contribute to the change that occurs in the chloride content of blood after death. When freshly drawn whole blood is stored in a test tube it will be found that there is a progressive shift of chlorides from plasma to cells. Imbibition by erythrocytes may account for a drop in plasma chlorides by as much as 10 per cent in 48 hours (Jetter and Moritz). It is apparent, however, that a reduction in the total chlorides of whole blood can only be accounted for by their diffusion out of the vessels and into the surrounding tissues. In life the antemortem chloride content of the tissues is much lower than that of the plasma. Thus the chloride content of heart muscle is approximately 50 per cent and that of skeletal muscle approximately 20 per cent of that of the plasma. On the other hand the magnesium content of cellular fluid is many times greater than that of plasma (Shohl). During life the maintenance of the chemical individuality of blood and tissues depends in part upon the ability of the cell membranes to regulate the diffusion of substances through them and in part upon the continuance of metabolic activity within cells whereby the integrity of their various organic constituents is preserved. After death not only do cell membranes become more permeable but the organic constituents of cytoplasm deteriorate so as to predispose to a free exchange of diffusible substances between the intra- and extracellular fluids in the direction of chemical homogeneity. Thus regardless of the cause of death the postmortem migration of chlorides out of the plasma and into cells and of magnesium out of the cells and into the plasma is to be expected. Data concerning the alterations that occur in the magnesium content of the blood incident to postmortem change as well as to drowning are presented in a later section of this review.

Blood chlorides (dogs) after drowning in fresh water. The blood chloride levels of 7 dogs drowned in fresh water are shown in table 6. Only in the first 2 were samples obtained from both the right and left sides of the heart. In the first dog the disproportionate reduction of chlorides in the left ventricle during the first 24 hours after death was greater than had been observed in any animal in the

control series. It is to be noted, however, that the greatest difference between the chloride levels in the two sides of the heart of this animal existed immediately after death and that the differences became progressively smaller as the postmortem interval lengthened. Although the chloride reduction was greater in the left, the right heart values were also depressed. Within 15 minutes after death the chlorides in the right heart were 52 mEq/L lower than in the antemortem sample. In no control animal was a reduction of this magnitude observed earlier than 24 hours after death.

In the second dog there was bilateral reduction in the chloride levels but the differences between the right and left sides of the heart did not exceed those encountered in control animals. The third dog also revealed evidence of bilat-

TABLE 6

Chloride content of blood of dogs taken before and at varying intervals after death by drowning in fresh water (milli-equivalents per liter)*

ANTE-MORTEM	POSTMORTEM INTERVAL	RIGHT HEART	LEFT HEART	POSTMORTEM DIFF. BETWEEN RH AND LH, LH−RH	DIFF. BETWEEN P.M. AND A.M. SAMPLES OF VENOUS BLOOD, P.M.−A.M.	REPORTED BY
128	¼ hr.	76	38	−38	−52	Jetter and Moritz
	12 hrs.	72	41	−31	−56	
	24 hrs.	64	42	−22	−64	
	3 days	49	40	−9	−79	
112	¼ hr.	96	99	3	−16	Jetter and Moritz
	6 hrs.	83	86	3	−29	
	24 hrs.	76	68	−8	−36	
108	0 hr.	71			−37	Guislain
102	24 hrs.	55			−47	Guislain
104	3 days	44			−60	Guislain
108	7 days	42			−66	Guislain
106	12 days	39			−67	Guislain

* Values designated * represent whole blood. All other values are for serum.

eral dilution of heart's blood but the low chloride values of the remaining animals could as well be attributed to postmortem change as to drowning.

Although the available experimental data concerning blood chloride concentration in dogs after drowning in fresh water are not sufficiently numerous to draw conclusions regarding the extent to, or the frequency with, which drowning in fresh water may result in pathognomonic change, it is clear that a sufficient degree of reduction may occur in the left side of the heart to be of diagnostic significance. It is also clear that unless the blood is obtained soon after death (within 24 hrs.) differences that might otherwise be of diagnostic value are likely to be masked by postmortem diffusion.

Blood chlorides (dogs) after drowning in salt water. The chloride concentrations of the blood of 13 dogs before and after death by drowning in salt water are shown in table 7. In all instances in which the samples were collected within the first 24 hours after death the serum chlorides were not only disproportionately high in

the left side but the differences between right and left sides were greater than had been observed in any animals of the control series. In the first 3 dogs the elevation was bilateral whereas in the last 10 chlorides were increased only in the left side.

In animals that were sampled repeatedly during the postmortem interval (1, 2, 3) it was observed that the disparity between the chloride concentrations

TABLE 7

Chloride content of blood of dogs taken before and at varying intervals after death by drowning in salt water (milli-equivalents per liter)

ANTEMORTEM	POSTMORTEM INTERVAL	RIGHT HEART	LEFT HEART	POSTMORTEM DIFF. BETWEEN RH AND LH, LH–RH	DIFF. BETWEEN P.M. AND A.M. SAMPLES OF VENOUS BLOOD, P.M.–A.M.	REPORTED BY
serum	*hrs.*	*serum*	*serum*			
123	¼	136	151	15	15	Jetter and Moritz
	6	114	161	47	−9	
	12	112	169	57	−11	
	48	95	96	1	−28	
123	12	139	152	13	16	Jetter and Moritz
	24	110	135	25	−13	
	48	107	119	12	−16	
	72	108	114	6	−15	
124	12	132	154	22	6	Jetter and Moritz
	24	95	123	28	−29	
	48	93	104	11	−31	
	72	91	96	5	−33	
whole blood		*whole blood*	*whole blood*			
104	0	101	141	40	−3	Tarsitano
103	0	99	143	44	−4	Tarsitano
106	0	109	131	22	3	Tarsitano
101	0	99	137	38	−2	Tarsitano
107	0	103	142	39	−4	Tarsitano
103	0	102	144	42	−1	Tarsitano
106	0	103	143	40	−1	Tarsitano
108	0	108	130	22	0	Tarsitano
102	0	102	141	39	0	Tarsitano
98	0	98	137	39	0	Tarsitano

in the right and left sides of the heart increased during the first 24 hours and decreased thereafter. Despite the fact that the disproportion in the chloride concentration in the two sides of the heart became less pronounced after 24 hours, its mean concentration remained higher than that of the control animals.

Blood chlorides (man) after death from causes other than drowning. Chloride values for right and left heart's blood of 46 persons dead of causes other than

drowning are given in table 8. In 21 the interval between death and sampling was stated. The other 25 were reported without specifying the time between death and autopsy. Although a variety of causes, both natural and violent, were represented, there was no consistent relationship between the cause of death and the rate of postmortem change in the chloride concentration.

If 99 mEq/L be regarded as the antemortem lower limit of normal for chlorides in serum, and 77 for whole blood (Gram; Peters and Van Slyke), it is apparent that a reduction in blood chlorides is a common if not a constant postmortem phenomenon. In 90 per cent of the cases in which postmortem samples of serum were analyzed the chloride level in one or both sides of the heart was below the lower antemortem limit of normal whereas in less than 25 per cent of the cases in which whole blood was analyzed did the concentration fall below the antemortem range.

That neither the intravascular shift nor the extravascular diffusion necessarily progress at the same rate on the two sides of the heart is indicated by the frequency with which dissimilar right and left heart values were encountered. Thus it may be seen in table 8 that the left heart serum values ranged from 7 milli-equivalents lower to 14 milli-equivalents higher than their corresponding right heart values. The chloride values for whole blood from the left heart ranged from 13 milli-equivalents lower to 8 milli-equivalents higher than their corresponding right heart values. A disparity as great as 8 mEq/L may develop within the first 5 hours after death from causes other than drowning. If diagnostic significance is to be attached to a difference in the chloride content of right and left heart's blood in cases of suspected drowning the difference should exceed those observed after comparable postmortem intervals in persons dead of causes other than drowning.

Blood chlorides (man) after drowning in fresh water. The results of chloride determinations on blood samples obtained at postmortem examination from the bodies of 34 persons presumably dead of drowning in fresh water are shown in table 9. In 14 information was available concerning the length of the interval between death and sampling. In 20 the analytical results were reported without specific reference to the length of the postmortem interval.

In 26 of the 34 cases blood was taken from both sides of the heart. In only one of these was the interval between drowning and sampling stated to be less than 12 hours. In this case autopsy was performed approximately 8 hours after death and the analysis disclosed a greater relative reduction in left heart chlorides than had been observed in any of the control cases. In the other 13 cases of right and left heart sampling in which the length of the postmortem interval was stated, the differences did not exceed those encountered in the control series. It may or may not be significant that in all 13 the left heart chloride levels were lower than those in the right.

It is difficult to assess the significance of blood chloride values in the absence of information concerning the interval between death and sampling. Soutter stated that in none of his cases was the interval between death and autopsy greater than 6 days. In one of Soutter's cases of fresh water drowning, blood

TABLE 8

Postmortem changes in the chloride concentrations of human blood

Deaths from causes other than drowning (milli-equivalents per liter)

SERIES I—POSTMORTEM INTERVAL STATED								SERIES II—POSTMORTEM INTERVAL NOT SPECIFIED				
Post-mortem interval	Whole Blood			Serum			Reported by	Post-mortem interval	Whole Blood			Reported by
	RH	LH	L-R	RH	LH	L-R			RH	LH	L-R	
hrs.												
5	89	88	−1	100	108	8	Moritz and McLean	?	83	83	0	Gettler
12				100	94	−6	Moritz and McLean	?	86	85	−1	Gettler
12	90	89	−1	92	101	9	Soutter	?	84	84	0	Gettler
16				78	77	−1	Moritz and McLean	?	84	84	0	Gettler
18	88	96	8	96	110	14	Moritz and McLean	?	77	76	−1	Gettler
18	78	65	−13	90	90	0	Moritz and McLean	?	81	80	−1	Gettler
18	81	85	4	96	89	−7	Moritz and McLean	?	95	95	0	Gettler
18				80	90	10	Soutter	?	87	87	0	Gettler
18	90	88	−2	95	90	−5	Soutter	?	84	84	0	Gettler
20	59	59	0		59		Moritz and McLean	?	82	81	−1	Gettler
22				107	104	−3	Moritz and McLean	?	120	119	−1	Gettler
24	87	84	−3	92	95	3	Moritz and McLean	?	85	84	−1	Gettler
24	86	86	0	91	90	−1	Moritz and McLean	?	82	82	0	Gettler
24				69	77	8	Soutter	?	85	84	−1	Gettler
24	59	59	0	62	62	10	Moritz and McLean	?	90	89	−1	Gettler
25	72	70	−2				Soutter	?	77	78	1	Gettler
25	77	82	5				Soutter	?	58	58	0	Gettler
30	84	84	0	84	86	2	Soutter	?	88	88	0	Gettler
30	86	84	−2	90	86	−4	Soutter	?	121	120	−1	Gettler
36	72	76	4				Soutter	?	101	100	−1	Gettler
36				56	51	−5	Moritz and McLean	?	92	92	0	Gettler
								?	90	88	−2	Gettler
								?	63	62	−1	Palmer and Doherty
								?	88	75	−13	Palmer and Doherty
								?	68	63	−5	Palmer and Doherty

TABLE 9

Chloride concentrations of blood of human subjects probably dead of drowning in fresh water (milli-equivalents per liter)

POST-MORETM INTER-VAL	WHOLE BLOOD			SERUM			REPORTED BY	POSTMORTEM INTERVAL	WHOLE BLOOD			SERUM			RE-PORTED BY
	RH	LH	L-R	RH	LH	L-R			RH	LH	L-R	RH	LH	L-R	
days															
½				99	76	−23	Moritz and McLean	<6 days	80	80	0				Soutter
½	92	89	−3				Stockis	<6 days	58	80	22				Soutter
1				55	56	1	Moritz and McLean	<6 days	66	82	16				Soutter
1	74						Müller	<6 days				95	89	−6	Soutter
2	74	68	−6	88	82	−6	Moritz and McLean	<6 days				78	80	2	Soutter
2	114	113	−1				Stockis	<6 days				72	80	8	Soutter
2-3	52						Müller	<6 days				86	82	−4	Soutter
3	65	58	−7				Moritz and McLean	<6 days				88	88	0	Soutter
4-5	54						Müller	<6 days				85	88	3	Soutter
4-5	80						Müller	<6 days	70	78	8				Soutter
5	54						Müller	<6 days	67	64	−3				Soutter
5-6	79						Leclerc	Unspecified	107	85	−22				Gettler
8-10	60						Müller	Unspecified	74	63	−11				Gettler
30	54						Müller	Unspecified	92	85	−7				Gettler
<6	58	58	0				Soutter	Unspecified	?	?	−1				Palmer
<6	70	68	−2				Soutter	Unspecified	?	?	−3				Palmer
<6	76	71	−5				Soutter	Unspecified	?	?	−3				Palmer

from the left heart contained 22 milli-equivalents more chlorides per liter than did the blood from the right side. Not only did this difference exceed any of those encountered in the control group but it was the reverse of what one would be led to expect from the results of animal experimentation. In general Soutter's findings in deaths presumably due to fresh water drowning are at variance with those of other observers.

Until chloride determinations made within 12 hours after death are available from a larger series of human cases of fresh water drowning final judgment regarding their value must be held in abeyance. Although there is adequate evidence from experimental animals (rabbits) of the diagnostic value of blood chloride determinations immediately after death from drowning in fresh water, it is clear from data derived from dogs and man that values obtained 12 or more hours after death are frequently of little or no diagnostic value.

In a human body recovered from fresh water a disproportionate depression of the chlorides in the left side of the heart of 17 milli-equivalents or more per liter should probably be regarded as presumptive evidence of drowning. Failure to find a significant postmortem difference in the chloride content on the two sides of the heart of such a body should not be regarded as evidence that death resulted from causes other than drowning.

In 8 of the 34 cases included in table 9 samples were taken from only the right side of the heart. Although the chloride values for these samples are lower than would be expected during life they are not significantly lower than those frequently observed in persons dead of causes other than drowning.

Blood chlorides (man) after drowning in sea water. Chloride determinations on samples of right and left heart's blood in 32 persons thought to have died of drowning in sea water are included in table 10. If 90 mEq/L of chloride represents the upper antemortem limit of normal for whole blood and 108 for serum (Gram; Peters and VanSlyke) abnormally high chloride values were present in over 80 per cent of persons dead of drowning in salt water. Another significant feature of the data shown in table 10 is that in 19 of the 32 cases of drowning in salt water the chloride level was not only higher in the left heart but the differences between the right and the left heart were greater than had been observed in any of the control series. It appears, therefore, that postmortem change is less likely to mask or simulate the effects of drowning in salt water than those of drowning in fresh water. It may be inferred from the published data that a preponderance of chlorides in blood from the left side of the heart of 17 mEq. or more per liter constitutes presumptive evidence of drowning in salt water.

Postmortem chloride content of pleural, pericardial, and peritoneal blood. In an effort to determine whether drowning leads to disproportionate dilution of fluids other than blood Guislain compared the postmortem chloride values of blood and pleural fluid of 5 dogs killed by cerebral trauma with those of 5 dogs that had been drowned in fresh water. Both the blood and the pleural fluid of the drowned animals showed lower chloride values than were observed in the controls and the chloride reductions in the pleural fluid were of the same order of magnitude as those of the blood.

Analyses of pleural, pericardial, and peritoneal fluids of human subjects dead of causes other than drowning are shown in table 11. Insofar as it is possible to interpret these data it appears that postmortem loss of chloride occurs most rapidly from peritoneal fluid, less rapidly from the blood and pleural fluid and least rapidly from pericardial fluid.

Chloride values for body fluids of human subjects dead of drowning in fresh water are shown in table 12. Although it was stated that none of these individuals had been dead longer than 6 days the absence of more definite information concerning the time of death makes it difficult to assess the significance of the

TABLE 10

Chloride concentration of blood of human subjects probably dead of drowning in salt water (milli-equivalents per liter)

POSTMORTEM INTERVAL	WHOLE BLOOD			SERUM			REPORTED BY	POSTMORTEM INTERVAL	WHOLE BLOOD			SERUM			REPORTED BY
	RH	LH	L-R	RH	LH	L-R			RH	LH	L-R	RH	LH	L-R	
days															
½	102	114	12	118	137	19	Moritz and McLean	Unspecified	86	95	9				Gettler
¾				92	113	21	Moritz and McLean	Unspecified	96	105	9				Gettler
1				109	134	25	Morits and McLean	Unspecified	111	124	13				Gettler
3	98	139	41	97	139	42	Moritz and McLean	Unspecified	99	103	4				Gettler
4	64	91	27				Gettler	Unspecified	81	81	0				Palmer and Doherty
5				87	87	0	Moritz and McLean	Unspecified	81	100	19				Palmer and Doherty
6	43	65	22				Gettler	Unspecified	100	111	11				Palmer and Doherty
Unspecified	41	47	6				Gettler	Unspecified	112	116	4				Palmer and Doherty
Unspecified	79	100	21				Gettler	Unspecified	88	128	40				Palmer and Doherty
Unspecified	105	116	11				Gettler	Unspecified	71	81	10				Palmer and Doherty
Unspecified	115	167	52				Gettler	Unspecified	91	176	85				Palmer
Unspecified	105	125	20				Gettler	Unspecified	?	?	34				Palmer
Unspecified	107	114	7				Gettler	Unspecified	?	?	34				Palmer
Unspecified	95	103	8				Gettler	Unspecified	?	?	15				Palmer
Unspecified	111	126	15				Gettler	Unspecified	?	?	17				Palmer
Unspecified	90	96	6				Gettler	Unspecified	?	?	33				Palmer

analytical results. Chloride levels of pleural fluid as low as 76 mEq/L had been observed in the control series, and in 5 of the 11 cases of fresh water drowning, in which pleural fluid was tested, chloride levels lower than 76 were encountered. The lowest chloride level observed in the peritoneal fluid in the control series was 67 mEq/L and in 3 of the 7 drowned persons chloride values lower than 67 were encountered. The lowest chloride level observed in the pericardial fluid in the control series was 84 mEq/L and in 2 of 9 drowned persons lower values were encountered. It is obvious that the control data are as yet inadequate to draw any conclusions concerning the significance of the chloride content of pleural, pericardial, peritoneal or pulmonary fluid in the diagnosis of death by drowning.

The significance of the magnesium content of the blood in the diagnosis of drowning in sea water. The significance of changes in the magnesium content of the blood in recognizing death by drowning in sea water was investigated by Jetter and

TABLE 11

Chloride concentration of body fluids of human subjects

Deaths from causes other than drowning.

Reported by Soutter (milli-equivalents per liter)

All specimens obtained after death

POSTMORTEM INTERVAL	BLOOD RIGHT HEART	PLEURA	PERICARDIUM	PERITONEUM
hrs.				
12	90		120	
16	70		90	80
18	90		108	87
23	69	76	94	67
25	77		85	74
25	72	85	96	
30	68	78	84	72
30	84	88	96	88
30	86	102	104	87
36	72	86	100	

TABLE 12

Chloride concentration of body fluids of human subjects after death from drowning in fresh water

Reported by Soutter (milli-equivalents per liter)

POSTMORTM INTERVAL	BLOOD RIGHT HEART	PLEURA	PERICARDIUM	PERITONEUM	LUNG
?	58	48	52	50	46
?	70	68			70
?	76				71
?	80		102		90
?	58	62	74		74
?	66	88	96	62	
?	95*	82	108	84	
?	78*	78	108	84	
?	70	69	98	62	
?	72*	80	96	84	
?	86*				70
?	88*	83	98	94	
?	85*	87			
?	67	49			

* Serum.

Moritz. In samples obtained from dogs dead of causes other than drowning it was observed that there is a progressive increase in serum magnesium beginning between 6 and 12 hours after death and continuing on to putrefaction at which

time values 8 to 10 times as high as the antemortem levels were reached. As in the case of postmortem chloride reduction the postmortem increase of magnesium in the blood does not always occur at the same rate in the two sides of the heart and differences between right and left heart as great as 0.7 mEq/L were observed.

After drowning in sea water there was an early and sharp rise in the serum magnesium concentration on the left side of the heart to levels considerably higher than had been encountered in any of the control animals short of putrefaction. Differences between the right and left heart's blood of animals drowned in salt water as great as 16 mEq/L were encountered.

The highest serum magnesium values observed by Moritz and McLean in 6 human subjects dead less than 72 hours of causes other than drowning was 7 mEq/L, whereas in 2 or 3 cases of salt water drowning examined within 24 hours after death serum magnesium values in the left heart of 22 and 23 mgm. per liter respectively were found. The greatest difference in the magnesium content of serum from the right and left sides of the heart of persons dead of causes other than drowning was 0.7 mEq/L and differences as high as 8 mEq/L were observed in all three of the cases of salt water drowning in which the blood was analyzed for magnesium.

If subsequent data concerning the rate and character of the postmortem changes in the magnesium content of blood conform to those now available it would appear that a preponderance of magnesium in the blood from the left side of the heart in excess of 1 mEq/L would constitute presumptive evidence of drowning in salt water.

Absence of significant chemical change in the blood of persons presumably dead of drowning. As already indicated the absence of chemical evidence of drowning can frequently be explained on the basis of postmortem artefact. Occasionally, however, and even though the autopsy is performed within a few hours of death, it is found that a person without demonstrable disease has unexpectedly died in the water without inhaling a sufficient amount of water to be recognized in the air passages, to produce changes in the lungs, or to cause significant alteration in the chemical composition of the blood. In such instances neither pathological nor chemical examination is likely to aid materially in establishing the cause of death. If drowning be defined as suffocation due to the inhalation of fluid such deaths might better be characterized as having resulted from syncope. Although it is beyond the scope of this review to discuss the possible mechanisms of such deaths it is pertinent to call attention to their occurrence. There are no reliable data from which the frequency of their occurrence can be estimated. It is the author's impression that unexplained (not due to inhalation of fluid) deaths in the water constitute considerably less than 10 per cent of all of the so-called deaths by drowning.

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THE RÔLE OF THE ADRENAL CORTEX IN PHYSIOLOGICAL PROCESSES

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The volume of literature dealing with various aspects of adrenal cortical function renders any review necessarily incomplete, both with respect to topics treated, and to references cited. This paper is restricted to a discussion of but certain features of cortex function and the reviewers have arbitrarily omitted reference to the interrelationships between the cortex and other endocrine glands, and to histological changes which the adrenal undergoes under various experimental conditions. Much of this literature has been covered in recent reviews (142, 460, 429, 274, 276, 211, 510).

No attempt has been made to survey work done prior to 1930 since this has been done by Britton (29). However, a few articles which cover the main trend of adrenal cortex physiology up to that time are included in the bibliography (425, 426, 12, 16, 134, 424, 182, 171, 164, 167, 342, 446, 447). Purely chemical problems concerned with preparation of cortical extracts and the isolation and identification of the various crystalline adrenal hormones also lie outside the province of this paper, and have been adequately discussed in several reviews (235, 236, 341, 399, 350, 351, 74). Probably most adrenal steroid chemists are agreed that not all of the hormones of the cortex have been isolated, for the amorphous fraction, which remains after the known steroid hormones have been removed from extract concentrates, is very potent in so far as life maintenance is concerned (235, 341, 249, 351).

For purposes of physiological treatment, the hormones of the adrenal cortex can be classified into two groups: 1, those closely allied to progesterone, the most active of which is desoxycorticosterone; and 2, the steroids which have an oxygen at the C-11 position, either in keto or hydroxy form, whose nomenclature is based on their relation to corticosterone. The physiological activity of these latter hormones, while showing quantitative differences, is essentially similar, and seems primarily concerned in the regulation of some aspects of organic metabolism as opposed to inorganic. For sake of brevity, we shall refer to this group as the corticosterones, except where it is necessary to discriminate between its members. Desoxycorticosterone is apparently the most potent of all known cortical hormones in maintaining the life of adrenalectomized animals, and in the control of certain phases of electrolyte metabolism. Yet it is present in but minute quantities in the adrenal, so that experimental results based on its use may eventually prove atypical.

A. *Factors which affect the life span after adrenalectomy.* Most adrenal work has been done on the dog, cat or rat. The guinea pig and rabbit present operative difficulties which preclude their extensive use. For many years it seemed

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that in the rat, unlike the other forms, adrenalectomy was fatal in only a fraction of the cases. The current view is that widespread accessory adrenal bodies may take over the function of the extirpated glands in this form, although such accessory bodies have not been clearly demonstrated to be frequent enough to maintain life. The mortality rate apparently depends in part on the strain of the animal (119, 121, 56). Removal of considerable surrounding tissue, said to be rich in accessory tissue, along with the adrenal glands, will increase the mortality (112, 339, 106, 142). If the animal is tided over the first few days following operation, thus presumably allowing time for accessory body hypertrophy, the mortality is decreased (129).

In the dog, the surgery involved in adrenal removal must be carefully performed, for injury to nerve plexuses adjacent to the glands will precipitate a rapid circulatory failure (243, 114). Adrenalectomy is therefore usually done in two stages (364, 114), a week or ten days elapsing between operations. This is unnecessary, however, if spinal anesthesia is employed (243, 106), or thorough procaine infiltration of the gland and adjacent nerve elements is performed previous to extirpation (243).

Young rats tend to have a shorter survival span than older animals (250, 106, 491, 418). Although there is some dispute on the point, the sex of the animal probably has no great influence on survival (364, 142, 418). In hibernating forms the season of the year materially affects the life span, the animals generally surviving the period of torpor (30).

The diet is extremely important in an assay based on the survival of adrenalectomized animals. The chief factor seems to be the intake of sodium chloride (373, 269, 159, 251, 128, 359, 491, 2, 6, 47), and the ratio of fed sodium to potassium. For reasons not clearly understood, a diet containing bread will increase the survival time of adrenalectomized rats (430, 56, 59). As will be discussed more fully later, environmental temperature must be carefully controlled.

It was first demonstrated by Rogoff and Stewart that the pregnant female dog (366) could survive removal of both adrenal glands. Further work showed that pseudopregnancy would also alleviate the symptoms of adrenal insufficiency (367, 445, 62). Whereas administration of estrogens shortens the survival period (45, 73, 403, 340), apparently anterior pituitary hormones (92, 45) and the corpus luteum hormone, progesterone, (122, 125, 386, 107, 494, 77, 139, 68, 91, 340, 67) can maintain life.

Many studies of assays for adrenal cortical hormones, based on the maintenance of life in adrenalectomized animals, have been published, for the dog (157, 343, 482), the cat (183), guinea pig (416, 381, 47) and the rat (188, 339, 418, 385, 44, 26, 78, 143). By their use, each steroid hormone has been tested. In general, desoxycorticosterone is the most potent (466, 298, 143, 91, 248, 340, 58, 357, 47), although the amorphous fraction is almost comparable (237, 238, 249). Larger amounts of the corticosterones are required (235, 248, 298).

B. *The relation of the adrenal cortex to electrolyte metabolism.* 1. *Sodium metabolism.* Baumann and Kurland (16) first called attention to a fall in plasma sodium and chloride, and a rise in potassium, which followed adrenalectomy in

cats, and expressed the opinion that the adrenal was concerned in sodium metabolism. Later it was shown that the injection of various sodium salts (292) or large amounts of Ringer's solution (365) would greatly prolong the life-span of adrenalectomized animals, and even revive such animals from acute insufficiency.

The importance of these observations was not fully realized until Loeb (268) showed that a significant decline in plasma sodium was characteristic of crisis in Addison's disease, and that the patient could be kept symptom-free by the administration of large amounts of sodium chloride (269). In careful metabolic experiments, Loeb and associates (273, 275) and, later, Harrop and co-workers (158) demonstrated that the decline in plasma sodium levels in the adrenalectomized dog was a reflection of an abnormally large excretion of this ion by the kidney. Adequate amounts of cortical extract restored the electrolyte pattern of the blood to normal, and prevented the renal wastage of sodium and chloride (156, 154, 158).

That sodium salts alone can maintain the life of adrenalectomized animals has been adequately demonstrated. Swingle and associates (450) were only partially successful in increasing the life-span by feeding sodium chloride to the point of tolerance. Harrop and co-workers (159) showed, however, that adrenalectomized dogs could be maintained for long periods if a sodium bicarbonate-sodium chloride mixture was given by stomach sound. Allers (1) and Allers and Kendall (2) devised the most successful therapy for maintaining adrenalectomized dogs, based on the feeding of sodium chloride–sodium citrate mixture with a diet low in potassium. Dogs were maintained on this regime, without cortical extract, for as long as 115 days, with normal values for all blood constituents. When the salt therapy was discontinued, the animals rapidly developed adrenal insufficiency. Cleghorn and associates (53) have confirmed these findings. With careful control of the mineral constituents of the diet, an adrenalectomized dog receiving no other form of treatment can be maintained without food for as long as seven days, which is a most severe test (237).

Sodium chloride administration will also maintain the adrenalectomized rat as shown first by Rubin and Krick (373) and Gaunt (128). Very large amounts of sodium chloride, however, may be injurious, and lead to death of the animal (8).

Stahl and associates (421) successfully treated numerous Addison's disease patients with sodium chloride alone, and Harrop and associates (163), on the basis of their own work and that of Loeb, suggested the use of a salt-free diet as a diagnostic test for patients suspected of having this disease. The evidence cited conclusively demonstrates that an adequate salt therapy can maintain normal health and normal blood chemistry both in adrenalectomized animals, and in patients with Addison's disease, provided the individual is not subjected to unusual stress of any kind (236, 237), and so long as the food intake remains normal (277).

The principal deficiency of the adrenalectomized animal with regard to sodium metabolism seems to lie in an inability of the tubular cells of the kidney to reab-

sorb the ion from the glomerular filtrate (152). One theory advanced to explain this renal failure was that increased sodium excretion was related to a decreased ability of the kidney of the adrenalectomized animal to produce ammonia (227), but this view has not been generally accepted.

When cortical extract is injected into the patient with Addison's disease, the normal human subject, the adrenalectomized dog, or the intact dog, the excretion of sodium is decreased, and that of potassium increased (464, 471, 180, 181). The sodium excretion is closely proportional to the dosage given (155, 160), which renders this technique valid for a bioassay of cortical materials.

The most potent adrenal hormone possessing an action causing renal retention of sodium is desoxycorticosterone (468, 474, 237, 493, 274, 247, 470). In some cases the action of this steroid on sodium excretion is so powerful that serum sodium concentrations may actually be elevated above normal (101, 348), and the serum potassium levels lowered to the point at which toxic symptoms appear (247, 101).

The corticosterones are less active in causing sodium retention, or, as in the case of 17-hydroxy-11-dehydrocorticosterone, actually increase the sodium loss (469). However, the renal effect is not specific for adrenal cortical hormones, since the crystalline sex hormones also cause sodium retention (472, 473, 154, 155, 239). Estradiol and progesterone are the most active of the sex hormones in this respect, testosterone the least active. Similar effects are obtained in the adrenalectomized animal, so that the renal action of the sex steroids is not mediated through the adrenal (467).

Hartman and his collaborators have shown that the action of cortical extract on sodium retention is gradually lost when injections are made intravenously over a period of time (165, 178, 180). This state of refractoriness does not develop when the extract is given subcutaneously (177). Refractoriness can be conferred upon a non-injected normal animal by the injection of serum from a refractory animal (477). However, the refractory state is not induced by injections of corticosterone, desoxycorticosterone or the precipitated sodium factor (173). Hartman and Lewis conclude that the factor necessary for sodium retention normally exists in a haptene combination with a protein contained in cortical extracts (173). It is apparently not identical with the factor which will maintain life in the adrenalectomized animal (176, 186, 185).

Absorption of electrolyte from the lumen of the intestine is distinctly abnormal in adrenalectomized animals (82, 422, 48), indicating that extra-renal defects may also be contributing to the impaired sodium metabolism.

Increases in intracellular hydration which should accompany depletion of extracellular sodium and chloride by renal wastage, have been repeatedly demonstrated in adrenalectomized animals (414, 411, 507, 344, 314). Muntwyler and associates (314) were able to correlate the gain in muscle water with the decline in extracellular sodium concentrations. The red blood cells show an increase in water content similar to that shown by muscle (194, 314).

Since an increased hydration of muscle and red blood cells also follows a lowering of extracellular electrolyte concentrations in the intact animal (81, 338,

521, 88, 314, 301), it can hardly be regarded as a specific response to lack of cortical hormones. Winter and Hartman (507) found, however, that skeletal muscle taken from adrenalectomized rats would gain water more rapidly in a hypotonic solution, and lose it more rapidly in a hypertonic solution than would tissue from intact rats. They attributed this to increased permeability of the muscle cells. Ponder and Gaunt (344) obtained negative results in similar experiments on rat muscles, but Angerer and Angerer (9) found the same changes noted by Winter and Hartman in muscles from adrenalectomized frogs.

As suggested by Swingle and associates (432), the actual hemoconcentration and fall in sodium concentration of the blood is often greater than that which can be accounted for by renal loss of water and sodium or by a shift of fluid into the intracellular compartment. Changes in membrane permeability are therefore suggested. It is now rather firmly established that the adrenalectomized animal in insufficiency does show intracellular electrolyte concentration changes. Voluntary muscles (194, 325, 153, 80, 314, 41, 51), cardiac muscle (80) and red blood cells (194) all show an increased potassium and a decreased sodium concentration. Liver cell potassium remains unchanged (80). These changes can be corrected by cortical extract injections, but similar shifts of ions in intact animals receiving cortical extract have not been obtained. Desoxycorticosterone injections will, however, strikingly alter the cation distribution in muscle cells. Muscle sodium is increased in both adrenalectomized and normal rats after prolonged treatment with this steroid (41, 306, 101) and intracellular potassium is reduced.

The intracellular electrolyte changes may not be reflecting a specific effect of adrenal cortical hormone upon membrane permeability, however. Nephrectomized rats show intracellular potassium increases similar to those of adrenalectomized animals, as the plasma level rises, indicating that the intracellular concentration is directly governed by the extracellular potassium level (80). Red blood cells of the normal animal (149, 150, 242) respond to changes in sodium and potassium concentrations of the plasma by shifts of water and of these ions across the cell membrane. When the cat or rabbit is subjected to depletion of extracellular sodium by intraperitoneal glucose injections, the red blood cells lose sodium into the plasma (361). Heppel (196) found that the muscles of the intact rat would gain sodium and lose potassium when the animal was fed a potassium-free diet. On the other hand, potassium injections will increase muscle potassium and lower muscle sodium (307). Thus while it is true that electrolyte shifts into and from the intracellular compartment occur in adrenal insufficiency, they apparently coincide with a fall in plasma sodium and a rise in plasma potassium concentrations, and are of the same magnitude as the intracellular changes exhibited by intact animals with similar extracellular electrolyte changes.

The observed reduction in intracellular sodium contradicts the assumption previously mentioned, that a plasma sodium loss greater than that which can be accounted for by excess renal excretion, can be attributed to a shift into the tissues.

It has been frequently observed that the plasma volume of untreated adrenalectomized animals (233, 517, 162, 448, 454) and of the patient with Addison's disease in crisis (371, 274) is very low. Cortical extract will restore the volume to normal, but will not raise it above normal. Desoxycorticosterone has a remarkable effect, however, in increasing the plasma volume above normal in the Addison's disease patient (274, 474, 102, 377), the normal human subject (60), and in the intact and adrenalectomized dog (438, 61). Clinton and Thorn (60), using normal subjects, found that sodium chloride given with the desoxycorticosterone did not materially increase the plasma volume over that obtained with the steroid alone. The increased volume is, however, apparently associated with a marked retention of sodium and chloride, for withdrawal of steroid treatment is followed by rapid elimination of the retained salt and water, and the plasma volume declines to normal.

It seems probable that more is involved in the effect of cortical hormones upon plasma volume than the simple production of a positive water balance. McAllister and Thorn (299) reported that cortical extract could prevent the reduction in plasma volume which follows prolonged etherization in dogs. Similarly, Ragan, Ferrebee and Fish (347) found that desoxycorticosterone prevented the small decrease in plasma volume associated with ether anesthesia and surgical procedures in patients. Their results with cortical extract were inconclusive, however.

Not only will desoxycorticosterone increase the plasma volume, but the arterial pressure is raised as well. Cortical extracts, even in massive doses, do not induce persistent or transient hypertension in either man or animals. Desoxycorticosterone, however, may cause hypertension in patients with Addison's disease, especially when the salt intake is high (377, 102, 419, 465). Reduction of dosage will cause the hypertension to disappear. Similarly, the blood pressure of the dog (247, 258, 438, 363) and rat (145, 28) can be elevated above normal. This hypertension cannot be directly correlated with plasma volume changes (438). The pressor action shown by desoxycorticosterone is also exhibited by many different steroids (145, 28). Grollman, Harrison and Williams (145) believe that the steroids have a toxic action upon the kidney and cause release of the pressor material, renin, but the evidence is indirect. Loeb (271) has suggested that, since in some patients with Addison's disease salt alone may elevate the arterial pressure above normal, the effect of desoxycorticosterone may be due merely to an ion effect upon the blood vessels. Swingle and co-workers have suggested that desoxycorticosterone may perhaps affect the tone of the arterioles (438).

2. *Potassium metabolism.* It was noted some years ago that removal of the adrenal glands was followed by a rise in the serum potassium level, so that comparatively high concentrations were reached by the time of death (16, 189). Later work showed that not only was the adrenalectomized animal exceedingly sensitive to administered potassium (530, 526, 3, 59, 480, 505), but that potassium salts given to the intact animal in quantities sufficient to raise the plasma con-

centrations to those typical of terminal adrenal insufficiency would reproduce many of the symptoms of the adrenalectomized animal, and might lead to death (189, 481, 530, 528, 531).

The therapeutic value of a diet low in potassium in the treatment of Addison's disease or for the maintenance of adrenalectomized animals has been repeatedly demonstrated (1, 2, 3, 325, 498, 378). Potassium administration has, in fact, been successfully used as the basis for a test for incipient adrenal deficiency in the human (76, 529).

Harrop, Soffer, Ellsworth and Trescher (158) first showed that the increase in plasma potassium concentrations was largely a reflection of a decreased renal capacity to excrete the ion. This kidney failure could be corrected by cortical extract (154, 158). Harrison and Darrow (152) attributed the renal dysfunction to a disturbance in tubular function, so that potassium was not concentrated in the urine in a normal manner. This potassium retention may not hold true for exogenous potassium, at least in the rat (380).

Not all investigators agree that renal failure will account for the whole of the plasma potassium increase found in adrenal insufficiency. Marenzi (290) found that injected potassium was fixed by the tissue cells less readily in adrenalectomized animals. Winkler and associates (505) also found that plasma potassium levels were elevated by a smaller amount of injected potassium than that required for the intact dog. Cortical extract is, according to Marenzi, concerned in binding potassium in the tissue cells, in regulating the potassium equilibrium between tissues and plasma, and in regulating the excretion of excess plasma potassium. Ingle, Nilson and Kendall (219) observed that cortical extract would retard the rise in potassium concentration and prolong life in the adrenalectomized-nephrectomized rat. Other investigators using this type of animal have reported either negative or inconclusive results insofar as potassium changes are concerned (284, 172).

Desoxycorticosterone has an action upon potassium metabolism even greater than that of cortical extract. Long continued administration of this steroid may lead, in the dog, to muscle weakness and intermittent paralysis, which is in large part attributable to the extremely low plasma potassium concentrations found (247, 101). The symptoms can be readily prevented by potassium injections. The use of a low potassium diet with desoxycorticosterone therapy in patients with Addison's disease may have serious consequences (478). The striking effect of desoxycorticosterone upon potassium metabolism is probably largely upon the kidney, for the steroid will cause an increase in potassium excretion even in the intact animal (247). However, Talbott and co-workers (458) found quite low potassium clearances in patients with Addison's disease even after long periods of desoxycorticosterone therapy.

Desoxycorticosterone markedly affects the passage of potassium into and out of the cells. It will prevent the usual increase in intracellular potassium which accompanies adrenal insufficiency, and will actually lower the tissue potassium in intact animals (306, 101, 41). Myocardial fibers of the heart show lesions

comparable to those observed in rats fed a potassium-free diet (306). This effect in reducing muscle potassium is also shown to some extent by the sex steroids (305).

The accumulation of potassium in muscle cells during adrenal insufficiency probably will not account for the asthenia characteristic of this condition (306). Similarly, the toxic effects of injected potassium appear to be directly related to elevation of the serum concentration, and only indirectly to the rise in muscle potassium (307). On the other hand, Ferrebee and associates (101) have suggested that the muscle weakness which follows prolonged treatment with desoxycorticosterone may be associated with the decreased muscle potassium, and its replacement with sodium.

The idea that accumulation of potassium in the serum may, in itself, account for the symptoms of adrenal insufficiency has not had wide acceptance. Keith and Binger (232) report that induced high levels of serum potassium in normal human subjects, and equally high levels in diseased patients, do not necessarily produce toxic symptoms. Schamp (382) was unable to obtain symptoms resembling those of adrenal insufficiency in normal dogs injected, for long periods, with potassium salts. The patient with Addison's disease may show little or no potassium retention even in crisis, and, on the other hand, may show greatly elevated serum potassium levels and yet remain symptom-free (271). Adrenalectomized animals dying of circulatory failure after trauma show no consistent serum potassium change (444, 355).

Neither can the cause of death following adrenalectomy be attributed solely to the upset in the metabolism of sodium, although this was at one time a rather common assumption (273, 272, 158). While renal wastage of salt and water and consequent dehydration certainly contribute to the fatal collapse of the adrenalectomized animal, a considerable body of evidence indicates that these changes, *per se*, may not be the primary factors involved. Experiments have been devised in which the serum electrolyte concentrations of adrenalectomized animals are reduced to levels as low as or lower than those characteristic of adrenal insufficiency, without producing any symptoms whatever. Such electrolyte depleted animals can be run through complete cycles of circulatory failure and subsequent restoration to normal health and vigor by withholding or administering cortical extracts (440, 442). Moreover, circulatory collapse in adrenalectomized dogs need not involve any change in blood sodium or in body water (444, 355). Intravenous injections of a strong hypertonic salt solution can restore the failing circulation and electrolyte concentrations of the dog in acute adrenal insufficiency, but the effect is temporary. Within a few hours the blood pressure again declines to a level incompatible with life, and the animal may die with serum electrolytes still well elevated above normal (439, 443).

Hartman, Lewis and Gabriel (175, 176) reported that adrenalectomized dogs rendered refractory to cortical extract insofar as renal retention of sodium is concerned can be maintained in apparently normal health by extract injections, while the plasma sodium is at the level characteristic of adrenal insufficiency.

Loeb (270) has reported cases of Addison's disease where the electrolyte pattern of the blood is virtually normal at death.

Some animal forms, such as the opossum (36, 412, 187) and the elasmobranch (174) show either no decrease or even an increase in plasma sodium during adrenal insufficiency.

Further evidence on this point is furnished by experiments employing the intraperitoneal injection of isotonic glucose (383, 81). Intact animals so treated show symptoms resembling, in many respects, those characteristic of adrenal insufficiency, which include low serum sodium concentrations, hemoconcentration, and decreased tolerance to stress (131, 361, 314). As has been frequently demonstrated, the adrenalectomized animal is extremely sensitive to intraperitoneal glucose injections (439, 154, 124, 515, 352).

In the adrenalectomized dog, the depletion of extracellular electrolyte and the dehydration of the blood is followed by a progressive decline in blood pressure and symptoms of acute circulatory collapse (439). Qualitative differences in the electrolyte transfers induced in adrenalectomized and intact animals by intraperitoneal glucose injection have not been observed. Circulatory failure can be prevented by either cortical extract or desoxycorticosterone (435), even though plasma electrolyte concentrations remain low (440). Blood chemistry studies made on adrenalectomized rats subjected to intraperitoneal glucose injections have revealed: *a*, that less electrolyte is shifted into the peritoneal cavity than in the normal rat; *b*, that, despite this smaller shift, serum electrolyte concentrations actually fall lower than in the normal rat; and *c*, the blood concentration is more severe (352). Either cortical extract or large amounts of desoxycorticosterone can prevent the fatal collapse, but neither will correct in entirety the abnormalities in electrolyte transfer (353). However, the electrolyte imbalance does not seem directly concerned in the production of circulatory collapse, except insofar as it throws a strain upon an asthenic peripheral circulation.

The mechanism underlying the decreased ability to transfer fluid and electrolyte from one body compartment to another is not clear. Cantarow and Rakoff (42, 349) found that desoxycorticosterone, in common with the sex hormones, increased the rate of chloride transfer from the blood to the peritoneal cavity in the dog, but a similar change is not observed in the rabbit (349) or rat (353).

C. *The relation of the adrenal cortex to renal function.* Aside from renal wastage of salt and water, and retention of potassium, in adrenal insufficiency, the adrenalectomized animal shows changes in blood and urine chemistry which are indicative of a serious impairment in renal function. There is an early rise in blood non-protein-nitrogen and urea nitrogen levels (23, 12, 414, 433, 434, 279, 182, 520, 160, 525), and an appreciable increase in blood sulfate (455, 509), creatinine, uric acid and phosphate levels (434, 522, 182, 160). In 1916 Marshall and Davis (297) showed that the urea retention of the adrenalectomized dog was associated with a decreased excretion of creatinine and phenolsulphonphthalein. A decreased urea clearance was also noticed by Bevier and Shevsky (23) in the

adrenalectomized rabbit. While a more or less acute lipoid nephrosis of the kidney tubules in terminal adrenal insufficiency has been described (12, 288, 146, 417) it now seems agreed that visible pathological changes are neither consistent nor widespread enough to explain the impairment in kidney function (433, 130, 297).

Stahl and associates (421) showed that the clearance of either urea or phenolsulphonphthalein was not reduced below normal in adrenalectomized animals so long as cortical extract or salt was given, but that lowered clearances followed shortly after the removal of maintenance therapy. A close correlation has been repeatedly drawn between the actual level of non-protein nitrogen in the blood and the onset of symptoms of adrenal insufficiency (520, 182, 160, 421, 449). The possibility that death in the adrenalectomized animal is due primarily to uremia is not tenable, however, for, as several workers have demonstrated (81, 300) blood urea nitrogen levels higher than those usually observed in terminal adrenal insufficiency are not necessarily lethal to the animal with intact glands. The blood urea changes are, however, indicative of a progressive renal failure, which must be regarded as an essential factor in the development of uncomplicated adrenal insufficiency.

Distinct deviations from normal in the renal excretion of nitrogenous waste products, of sodium, of potassium, and of water are now recognized as characteristic of adrenal insufficiency. At present, these various deficiencies do not seem to have a common denominator.

The inability of the kidney of the adrenalectomized animal to transfer sodium from the glomerular filtrate to the plasma, and also its inability to excrete potassium in normal quantities, have already been discussed. Both are supposedly due to a defect in the metabolism of the renal tubules, but one which can be corrected by desoxycorticosterone (468), and the amorphous fraction (238) so that it is presumably not directly linked to a disturbance in intermediary carbohydrate metabolism.

The decreased excretion of nitrogenous waste products in the adrenalectomized animal is probably related to extra-renal defects. The increase in blood urea nitrogen following adrenalectomy in the dog occurs simultaneously with the fall in blood pressure, and does not precede it (449). In fact, there is a good reciprocal correlation between the blood pressure and blood urea changes. Talbott and associates (458) have recently shown, however, that a deficiency in the rate of glomerular filtration exists in the patient with Addison's disease even when the blood pressure is well maintained, so that hypotension is certainly not the sole factor concerned. The sharp reduction in blood volume which follows reduction of sodium and chloride concentrations also must play a rôle in decreasing the renal blood flow in the adrenalectomized animal. Talbott and associates (458) suggest that a diminution in efferent arteriolar tone may play a significant part in the reduced urea clearance.

Desoxycorticosterone will increase the urea excretion, and lower the elevated blood urea levels (466, 274, 176, 377). In fact, the blood urea level of the normal animal can be lowered by administration of large amounts of this steroid (247,

357). Not all of this difference can be attributed to an increased rate of glomerular filtration, for desoxycorticosterone will also decrease the severity of uremia in nephrectomized animals (398, 362, 85, 404). Other indirect evidence indicates that this steroid may actually depress protein catabolism of the body (357, 355).

D. *Relation of adrenal cortex to water diuresis, water intoxication and diabetes insipidus.* In most, but not all, animals, the altered electrolyte excretion which follows adrenalectomy is associated with a diuresis. This subsides, as symptoms become severe, and terminally an oliguria or anuria ensues (162, 507, 449, 373, 19, 380, 126, 274). Yet despite this water diuresis associated with sodium loss, the ability of the kidney to excrete water shows deficiencies. If distilled water is administered by mouth, even in small doses, the diuretic response is far below normal (291, 118, 372, 260) and susceptibility to water intoxication is extreme (360, 441, 124, 98). This loss of the normal diuretic response to water is evident in the rat within 18 hours after adrenalectomy and later becomes more marked even in animals well-maintained on salt (118). Protection against water intoxication is also afforded the adrenalectomized rat to a certain degree by salt (441, 124).

Kottke and associates (246) observed no abnormality in a urine dilution test performed on adrenalectomized dogs maintained on salt. The ability to concentrate urine was defective, however, in hot weather.

Desoxycorticosterone shows activity in preventing water intoxication in adrenalectomized animals (453, 98), but whole gland extract or the corticosterones (98) are more effective. The sex hormones show no activity (125). Cortical extract and desoxycorticosterone will also provide a life-maintaining protection for normal rats against doses of water otherwise lethally intoxicating (118).

The mechanism underlying the loss of the diuretic response to water after adrenalectomy has not been fully elucidated, although several contributing factors are known. In the rat, delayed diuresis after water is given by mouth can be accounted for in part by a decreased stomach-emptying time and rate of intestinal absorption (118). The decreased absorption rate is probably associated with an abnormal electrolyte shift into the intestinal lumen (118, 422). However, either absorbed water (118, 361a) or injected fluid (360, 241) is not excreted at a normal rate in adrenalectomized animals, or in patients with Addison's disease. Further, intoxication symptoms appear at lower levels of water retention in adrenalectomized than in normal dogs (441). Water intoxication is apparently not similar to most other stresses in adrenalectomized animals, since, at least in its early stages, it is not associated with a marked fall in blood pressure (441, 118).

The hypophysectomized animal also shows a striking loss of diuretic response to water and a high susceptibility to water intoxication (124, 228), which can be relieved by desoxycorticosterone or cortical extract (228).

Large doses of desoxycorticosterone, when prolonged over a sufficient time interval, will produce a syndrome of polydipsia and polyuria similar to that of diabetes insipidus (348), except that pituitrin is ineffective and fluid restriction

does not cause dehydration. Withdrawing salt from the diet reduces the severity of the polyuria (312). An imbalance between the effect of the adrenocortical and posterior pituitary hormones upon the kidney has been postulated (312, 413). It is true that desoxycorticosterone (70, 400), unlike cortical extract (388), will produce a diuresis in hypophysectomized rats. However, the quantities of the steroid required to produce polyuria are so large that the effect may be of the nature of a simple overdosage phenomenon (387).

Another explanation for the polyuria following desoxycorticosterone therapy might be that as the extracellular sodium concentration is increased, and intracellular hydration is decreased, the water intake, through increased thirst, will also be considerably increased (348, 356, 132, 81). In other words, the polyuria would be merely reflecting a polydipsia.

E. *Influence of the adrenal cortex on organic metabolism.* The observation that the blood sugar level of adrenalectomized animals was often low, or even at hypoglycemic levels, was reported many years ago (24, 345). Later it was shown that the liver glycogen levels were even less stable than were the blood sugar concentrations (345, 71) but these important findings attracted little attention at the time. Britton and his colleagues (32, 34, 33, 415) in a survey of the symptoms which follow bilateral adrenalectomy, in a wide variety of species, were impressed with the relative constancy of an appreciable decline in body carbohydrate levels, evidenced by blood sugar, and liver, heart and muscle glycogen changes. The occasional entire lack of significant decline in blood electrolyte concentrations, and the frequency of the development of hypoglycemia, seemed to warrant the conclusion that death from adrenal insufficiency was a reflection of a faulty carbohydrate metabolism of the tissues of the body through depletion of carbohydrate levels.

However, many observers pointed out that the blood sugar might easily be in the normal range even at the time of fatal collapse (161, 330, 141, 364, 12) which led some to doubt that regulation of carbohydrate metabolism was the prepotent function of the adrenal cortex.

Several reasons for this apparent discrepancy in experimental results now seem clear. There appears to be a rather wide species difference in the tendency for the blood sugar to decline significantly after adrenalectomy. In the adrenalectomized cat, for example, the final collapse of adrenal insufficiency is often associated with a definite hypoglycemia (34, 528, 433, 33). The adrenalectomized dog, however, but rarely shows a decline in blood sugar levels (161, 330, 364, 12, 182). A part of this species difference is probably explicable on the basis of differences in eating habits.

The problem of the relation of the adrenal cortex to carbohydrate metabolism was reopened with the work of Long and associates (277). In agreement with other workers, they found that so long as adrenalectomized rats were maintained in good health by the administration of sodium salts, no abnormalities could be observed in the storage of carbohydrate. When, on the other hand, the adrenalectomized animal refused food, or when it was forced to fast, the liver glycogen showed a dramatic fall to extremely low levels, accompanied by less severe but

significant declines in muscle glycogen and blood sugar values. Not only could these changes be prevented by the administration of cortical extract, but the liver glycogen levels could be increased well above normal even in these fasted animals, thus confirming previous observations of Britton and co-workers (34).

An increase in liver glycogen levels in the fasted animal, either normal or adrenalectomized, indicated that the administered cortical extract was either inducing a marked shift in carbohydrate reserves from other tissues of the body to the liver, or that the extract was stimulating the conversion of other materials into liver glycogen. The fact that all known glycogen deposits were increased contradicted the possibility of a carbohydrate translocation after extract treatment. In careful metabolic experiments, it was observed that the increase in liver glycogen levels was accompanied by an increase in urinary nitrogen excretion, with the ratio of extra carbohydrate formed to extra nitrogen eliminated indicating that the glycogen increment could be entirely explained by a conversion of endogenous protein stores to carbohydrate. Coincident with the increase in nitrogen output there was a fall in body weight, without a comparable increase in metabolic rate.

Evidence advanced by several investigators tends to support this general thesis that the action of cortical hormone may be more concerned with the catabolism of protein than in the actual utilization of carbohydrate. Not only will adrenalectomy relieve the symptoms of severe diabetes in the pancreatectomized animal (168, 278, 203, 280), but, as the glycosuria is reduced and finally eliminated, the level of urinary nitrogen also decreases (277, 220, 278). Cortical extract administration will increase the degree of glycosuria and also increase the urinary nitrogen. When phlorhizin is given to the adrenalectomized rat, the excretion of glucose and nitrogen is but a fraction of that observed with the phlorhizin treated normal animal (439, 199, 94, 264, 492). The administration of cortical extract produces a marked loss in body weight, increases the glycosuria and also the amount of nitrogen excreted. Likewise the rise in liver glycogen, accompanied by an increase in urinary nitrogen, which follows a short period of anoxia, is prevented by adrenalectomy (94, 108, 265, but see 253), and can be restored with cortical extract injections.

While this relation of the urinary nitrogen excretion to the changes in liver glycogen level appears clear, it should be remembered that a decreased nitrogen output, accompanied by an elevation of blood non-protein nitrogen concentrations, is one of the earliest signs of adrenal insufficiency, and denotes a decreased urea clearance by a hypofunctional kidney (151, 227, 158).

It should not be inferred that a regulatory effect of the adrenal cortex upon the catabolism of protein can satisfactorily explain all the metabolic deficiencies observable in the adrenalectomized animal. The rapidity with which the liver glycogen level declines in a fasted adrenalectomized animal is an indication that the tissues are oxidizing carbohydrate at an abnormally rapid rate. Evans (95) noted that when an adrenalectomized rat was fed glucose, it stored less as liver glycogen and used a greater portion than would an intact rat. Conversely, Russell (375) found that the administration of cortical extract to a glucose fed rat

increased the proportion stored as glycogen, and decreased the amount oxidized by the tissues. Katzin and Long (230) found a lower R. Q. in such extract treated rats. The conclusion that abnormal amounts of carbohydrate are burned by the adrenalectomized animal is supported by the experiments of Thorn and co-workers (264, 476). These facts might be interpreted as indicating that cortical extract actually inhibits the oxidation of carbohydrate. When, for example, force-fed depancreatized rats are given cortical extract, the increased glucose excretion which follows cannot all be linked to protein catabolism (220). Also, the decrease in sugar excretion which follows adrenalectomy is not entirely related to the decreased conversion of protein to carbohydrate (220). An interesting possibility is the one suggested by Wells and Kendall (493), that cortical hormone specifically blocks the action of insulin. It is true that the adrenalectomized animal is extremely sensitive to insulin, and that it can be protected by the administration of cortical extract (226) or adreno-cortical transplants (431). Whether this antagonism to insulin is other than an indirect reflection of a more fundamental upset in carbohydrate or protein metabolism is not proven, but research along these lines is likely to prove fruitful.

It would seem that the precise rôle of the adrenal cortex in metabolism must await clearly drawn results obtained on a study of the intermediary processes by which carbohydrate and protein are utilized in the tissues themselves. Some suggestions have already been advanced, but any attempt to evaluate them would be premature. There are, however, several points at which cortical hormones could be acting in their influence on metabolic processes.

1. *Intestinal absorption.* The theory advanced by Verzar (485, 486, 487, 308, 309, 221) that the hormone of the adrenal cortex is essentially concerned in the maintenance of the enzymatic processes by which carbohydrate or fatty acids are phosphorylated, was largely based upon the observation that the absorption rate for glucose and fat was reduced immediately after adrenalectomy. In fact, the adrenalectomized rat was reported to have lost the differential between the absorption rate of glucose, which is phosphorylated in the intestinal mucosa, and xylose, which is not. The reduced intestinal absorption shown by the adrenalectomized animal was therefore comparable to that shown by the animal poisoned with iodoacetic acid (486, 221, 308).

Since the publication of this scheme, much evidence has been advanced which has, with little exception, failed to substantiate Verzar's claims. It is true that the absorption rate of glucose is lessened in the adrenalectomized rat (71, 4, 296, 204). On the other hand, when the animal is maintained on sodium salts, the glucose absorption is normal (83, 4, 49). One reasonable explanation offered is that the maintenance of a normal absorption rate depends directly upon the maintenance of a normal food intake. Fasted normal rats, sham operated and unilaterally adrenalectomized rats show a decreased glucose absorption rate of the same order as that found in adrenalectomized rats (296), so that changes present shortly after operation presumably reflect the period of inanition on the day of the operation. Considerable indirect evidence supports this conclusion, for it has been shown that liver glycogen and blood sugar levels show little

tendency to decline so long as the adrenalectomized animal is in good condition and eating normally.

The claim of Verzar and other workers (487, 256, 257) that the absorption rate of fat is retarded after adrenalectomy, and can be restored to normal with either cortical extract or flavine and phosphoric acid, has led to considerable controversy. Some investigators have confirmed the decreased absorption rate (18, 17), while others deny that the salt-fed adrenalectomized rat shows any deficiency in fat absorption (13, 15, 50, 14, 304, 427) or in the phosphorylation of fatty acids in the intestinal mucosa (427). Whether this discrepancy may be due to differences in technique is not settled (14).

There is, however, insufficient evidence to justify the suspicion that an inability to absorb either carbohydrate or fat from the intestine plays a significant rôle in the metabolic upsets which accompany adrenal insufficiency.

2. *The conversion of absorbed glucose to glycogen in the liver.* Available indirect evidence indicates that fed carbohydrate can be transformed into liver glycogen at a normal rate in the adrenalectomized animal (277, 7, 236). However, Britton and Corey (31) have presented direct evidence that cortical extract affects the ability of the liver to convert glucose into glycogen. The perfused liver of an adrenalectomized rat failed to show glycogen storage with a gum-saline-glucose perfusion medium, even when insulin was given. When, however, cortical extract was added to the perfusion medium, there was a striking and rapid rise in liver glycogen levels.

Seckel (394) had earlier shown that cortical extract would retard the rate of glycogenolysis in liver slices taken from adrenalectomized rats. This decreased rate of conversion of glycogen to glucose under cortical extract action was confirmed by Corey and Britton (69) using isolated livers. The conclusion would be that cortical extract not only enhances the conversion of glucose into glycogen, but retards the breakdown of liver glycogen to maintain blood sugar levels. The importance of this shift in the equilibrium between glucose and glycogen in favor of the glycogen cannot be assessed at present. It might explain the fact that cortical extract will increase the proportion of fed glucose which is stored as liver glycogen as against that which is oxidized by the tissues. However, it seems doubtful if all the known deficiencies in carbohydrate metabolism shown by the adrenalectomized animal can be explained on the basis of this reaction alone.

3. *Deaminization of amino acids.* A faulty deaminization of amino acids in kidney or liver could well explain the decreased ammonia and urea excretion found in adrenalectomized animals, and also the parallel increase in urinary nitrogen which accompanies the increase in glycogen stores following the administration of cortical extract. Such a failure of deaminization, at least for the kidney, has some experimental backing.

Jiminez-Diaz (227), impressed with the frequency of acidosis in Addison's disease patients, advanced the explanation that the kidney had become "asthenic," and no longer produced ammonia at a normal rate. Kidney slices from adrenalectomized cats showed just such a failure of deaminization. Russell and Wilhelmi (376), in extending this work, showed that kidney slices from adrenalec-

tomized rats did not deaminize either administered alanine or glutamic acid at a normal rate. Samuels and co-workers (379) had observed earlier that alanine fed to adrenalectomized rats resulted in less storage of liver glycogen than would be true of normal rats.

It is curious that this failure of ammonia removal could not be demonstrated for liver tissue. Evans (96) found a normal rate of deaminization for alanine in liver slices from adrenalectomized rats. Likewise, Koepf and associates (244) found a normal deaminization of glutamic acid in liver tissue.

The basis for this tissue difference in the ability to deaminize amino acids, is not clear. At least, until this point is clarified, it would not be safe to attribute the principal deficiency in protein and carbohydrate metabolism shown by the adrenalectomized animal to a failure in the enzymatic process involved in the liberation of ammonia.

4. *Conversion of keto and hydroxy acid to carbohydrate.* Considerable evidence has accumulated that keto acids, such as those remaining after deaminization of amino acids, cannot be transformed into carbohydrate at a normal rate after the adrenals have been removed. Buell, Anderson and Strauss (39) found a retarded conversion of lactic acid to glycogen in the liver of adrenalectomized rats. Similarly pyruvate and succinate do not seem to be transformed into glycogen at a normal rate in liver tissue deprived of cortical hormone (476, 244). When the animal is treated with large amounts of cortical extract, the conversion rate of pyruvate to glycogen is actually increased to greater than normal (244). On the other hand, kidney slices from adrenalectomized rats evidently form carbohydrate from succinic and pyruvic acid at a normal rate (376). This again may be due to a difference in tissue source.

There is also some evidence that the animal as a whole cannot form glycogen at a normal rate from fed keto acids after adrenalectomy. Lewis and associates (264) found that in phlorhizin treated rats, the transformation of lactic and pyruvic acids into glycogen proceeded at a slower rate after adrenal removal, but their results have been criticized by Kendall (235). Wells and Kendall (494) believe that there is no diminution in the ability to convert fed protein into liver glycogen in the phloridzinized adrenalectomized animal. Levy Simpson (263) has suggested that cortical extract increases the conversion of endogenous lactic acid to carbohydrate in the Addison's disease patient.

5. *Oxidation of glucose.* The previously mentioned experiments indicating that the adrenalectomized animal burns a greater amount of carbohydrate than does the cortical extract treated animal (95, 375, 264, 476) would not necessarily demand that the actual process of glucose oxidation was abnormal. If, for example, the catabolism of other endogenous energy yielding materials, such as protein, was impeded in the adrenalectomized animal, the oxidation of a larger amount of carbohydrate would necessarily follow.

The only direct evidence on the ability of the tissues from adrenalectomized animals to utilize carbohydrate has been obtained on voluntary muscle. It has long been recognized that muscle from the adrenalectomized animal rapidly loses its normal capacity to perform work. In fact, several accurate methods of bio-

assay of cortical principles are based on the restoration of the normal work capacity either of the whole animal (117) or of isolated muscles (212, 147, 97). Ingle, in a number of studies on the work capacity of the gastrocnemius muscle of adrenalectomized rats, has shown that asthenia develops shortly after extirpation of the glands, and that it is not corrected by injections of salt (213, 218). Injected glucose produces an improvement in the work capacity, approximately equivalent to that obtained by the injection of cortical hormone (218). Winter and Knowlton (508) attribute the asthenia to a metabolic deficiency of the muscle of the adrenalectomized animal. The fundamental nature of the decreased work capacity is, however, not known, for factors such as circulatory collapse and the decreased muscle glycogen have not been eliminated (218).

Britton and his associates (32, 34, 35, 33) have held that muscle glycogen levels are without exception seriously depleted in adrenalectomized animals, and conversely, that these glycogen levels could be restored by the administration of cortical extract. The present consensus of opinion is, however, that while muscle glycogen may show a decline after adrenalectomy only after food is refused (277, 375) muscle glycogen is not increased in the normal animal treated with cortical extract. Whether a carbohydrate deficiency other than that attributable to a lack of available glycogen is present in the muscle is uncertain. The working muscle does seem to produce less lactic acid when deprived of cortical extract (11, 315, 66). Also there is impairment of the ability of the gastrocnemius to produce lactic acid autolytically (40, 66). A decrease in total phosphorus and in inorganic phosphate following adrenalectomy has been reported (40). Some workers (252, 66) believe that there is a reduction in phosphocreatin, which would imply a derangement in the recovery process of muscle. Others doubt the significance of the phosphocreatin changes after adrenalectomy (281). Cope and co-workers (66) could find no difference in the enzymatic formation of lactic acid by muscle extracts from normal and adrenalectomized animals. They concluded, however, that less work could be performed because the muscle could no longer resynthesize phosphocreatin at a normal rate.

Verzar and Nontigel (488) found a decrease in glycogen phosphorylation in muscle after adrenalectomy, but since restoration was obtained with desoxycorticosterone, which does not, to an appreciable extent, influence muscle work (215), their result is difficult to evaluate. Nicholson and associates (323) showed that the ability to develop a delayed tension (5th stage of neuro-muscular transmission) after a motor nerve stimulation, was reduced after adrenalectomy and could be restored by desoxycorticosterone. There is some evidence that the energy capacity of the normal dog (87), rat (148), human (205, 310) and asthenic patients (136) may be increased by cortical extract therapy.

It is not unlikely, therefore, that a deficiency in work capacity is related in part to an inability of the muscle from an adrenalectomized animal to utilize efficiently available carbohydrate, but the nature of this deficiency remains elusive.

6. *Production and combustion of acetone body residues.* Just as adrenalectomy reduces the glycosuria of depancreatized animals, it also tends to lower or abolish the excretion of ketone bodies (278, 280). Similarly, adrenalectomy will abolish

the ketonuria following phlorhizin injections (95), pregnancy (282), fasting (282) and the administration of anterior pituitary extract (116, 283). The actual production of ketonuria by cortical extract is not so clearly established. Ketosis following the injection of 17-hydroxy-11-dehydro-corticosterone was obtained by Ingle and Thorn (220) in depancreatized rats, but, as Ingle (211) points out, this followed the development of a glycosuria sufficiently severe to be accompanied by ketonuria itself. Ketone body excretion is increased in phlorhizin treated adrenalectomized rats after treatment with corticosterone, but the condition of the animal is at the same time improved (235). If fat is fed the phlorhizinized animal, there is little increase in the degree of ketosis unless cortical extract is given (494). However, desoxycorticosterone, which seemingly lacks an effect on carbohydrate metabolism, is also effective in this regard. Ketonuria could not be produced in normal rats with cortical hormones (407).

A direct relation of cortical hormone to acetone body production following anterior pituitary extract is rendered unlikely by the observation of Mirsky and associates (311, 317) that there is no parallel decrease in the blood level of acetone bodies of adrenalectomized animals accompanying the decrease in ketonuria. This observation has since been confirmed (287, 408). An explanation would be that the hypofunctional kidney shows a higher threshold level for acetone bodies. Shipley (406), however, disagrees that the diminished ketosis in adrenalectomized rats treated with anterior pituitary extract can be explained solely on the basis of renal threshold level. In his experiments, ketonemia was reduced but not abolished, and ketonuria was likewise reduced but not necessarily abolished. Mirsky and associates (317) would agree that adrenalectomy does produce a reduction in the rate of ketone body formation and in the rate of utilization.

An interesting fact, of uncertain significance, is that the fatty infiltration of the liver which follows the administration of anterior pituitary extract (116, 282), or poisoning with phlorhizin (487) can be prevented by adrenalectomy. Adrenalectomy will also reduce the fat deposition in the regenerating liver of partially hepatectomized fasted animals (285). Cortical extract treatment allows the fatty infiltration to progress normally.

By way of summary, then, it would seem that cortical hormones may control carbohydrate metabolism by: *a*, increasing the conversion to glycogen of fed carbohydrate; *b*, allowing the conversion of endogenous protein to glycogen, assisting either in the process of deaminization of the amino acids, or in the conversion of the keto and hydroxy acids to carbohydrate; *c*, decreasing the oxidation of available carbohydrate.

In almost all cases where cortical extract has an effect on the physiological processes involved in carbohydrate and protein metabolism, identical or even greater effects can be obtained with the corticosterones, which can prevent the depletion of liver glycogen in fasted adrenalectomized animals (277, 137, 276), raise the resistance to insulin injections (137, 138), increase the work capacity of voluntary muscle (214), augment the conversion of glucose to liver glycogen (277) and increase the conversion of keto acids to liver glycogen (264). It is also true that the adrenal steroids lacking in oxygen at C-11, such as desoxy-

corticosterone, 17-hydroxydesoxycorticosterone, or the sex steroid progesterone (277, 235, 265, 215, 137), have little effect on any of these processes, although they may increase liver glycogen levels in some species (123, 72).

The ability to augment liver glycogen stores is not as specific for cortical hormones as once believed, however, for Janes and Nelson (224) showed that diethylstilboesterol would increase liver glycogen in the normal rat. This observation has been amply confirmed not only for this synthetic hormone but for the naturally occurring estrogens as well (140, 84, 216, 217). Accompanying the increase in carbohydrate is a rise in urinary nitrogen. Dolin, Joseph and Gaunt (84) found that estrogens would increase the glycosuria of depancreatized ferrets, and Ingle (216) reported that they would produce glycosuria in the sugar-fed normal rat. Long (276) found that diethylstilboesterol would not increase the liver glycogen level in either the adrenalectomized or hypophysectomized animal, and concluded that the action of the sex steroids was on the adrenal cortex via the anterior pituitary. Ingle (217) was able, however, to produce glycosuria in the adrenalectomized rat receiving relatively small amounts of cortical extract by prolonged sex steroid therapy. Death might follow with blood sugar levels still markedly elevated. This problem remains, therefore, unsettled.

The metabolic upsets in protein and carbohydrate metabolism found in the adrenalectomized animal are, in large part, present in less acute form in the hypophysectomized animal. No attempt has been made to review the rather extensive literature pertaining to the rôle of the anterior pituitary on organic metabolism, but it seems apparent that all known carbohydrate effects of the pituitary cannot be explained solely on the basis of its action in maintaining the functional integrity of the adrenal cortex (277, 276, 94, 375, 374, 21, 202, 429).

Evidence on the relation of the adrenal cortical hormone to vitamin metabolism is controversial. For example, evidence for (266, 267, 500) and against (483, 428, 144, 225) an influence on the synthesis of vitamin C has been presented. The conclusion of Verzar and Laszt (485, 486, 257) that phosphorylated riboflavine could maintain the adrenalectomized rat, has been repeatedly denied (295, 46, 316, 37, 100).

A defect in the calcification of teeth (337, 384) and bones (501) is present in the adrenalectomized animal, but its nature is obscure. Butcher (38) found that hair growth was accelerated after adrenal removal, which is opposite to the effect which might be expected on the basis of the hirsutism associated with adrenal tumors, and also the hair shedding which accompanies adrenal insufficiency in the dog. The hair shedding following adrenalectomy in the rat has been attributed to loss of the medulla (423). It is common observation, however, that hair coat changes can be partially corrected in desoxycorticosterone maintained adrenalectomized dogs.

F. *The adrenal cortex and resistance to stress.* An extensive list can be constructed of drugs, poisons, toxins, infections, altered environmental conditions, and traumatic procedures to which the adrenalectomized animal is highly susceptible. Only those which have received greatest attention can be discussed here.

1. *Drugs, poisons and other pharmacological agents.* Work has centered chiefly upon two, morphine (389, 479, 286, 259) and histamine (79, 293, 513, 516, 209, 369, 326). For both, the toxic dose for adrenalectomized animals is but a fraction of that required for the intact animal. The resistance can be readily increased by the injection of cortical extract, although it is questionable whether complete restoration to the normal is thereby attained and whether the adrenal medulla may not also play a part in the lowered resistance (513, 209), at least to histamine.

The simplest explanation for the lowered resistance would be that the capacity to destroy or inactivate the administered drugs was impaired. There is evidence that this may be the case for histamine (369). The capacity of histamine inactivation can be restored by cortical extract (368) and, less effectively, by desoxycorticosterone (368, 326).

If the secretion of the adrenal cortex is specifically involved in the inactivation of injected drugs, then it should be possible to enhance the resistance of the normal animal by cortical extract treatment. Perla and associates (334) did find that cortical extract would increase the efficacy of a saline infusion in preventing shock after histamine injections. The coupling of two therapeutic measures, either of which might be useful in treatment of shock, makes the results difficult to interpret. Irradiation of normal dogs with carbon arc lamps produces a fall in blood pressure. Graham (136a) observed that in adrenalectomized dogs the fall in diastolic pressure averaged 63 per cent. Both cortical extract and desoxycorticosterone prevented the blood pressure fall in untreated normal and adrenalectomized animals. The protective action of the steroid and extract was assumed to be due to the effect they exert against the toxic manifestations of histamine since carbon arc irradiation results in the release of a vasodilator substance from irradiated tissues.

2. *Toxins, anaphylaxis, infections.* The adrenalectomized animal is highly susceptible to but small amounts of diphtheria toxin (20, 197), typhoid vaccine (457), bacterial infection (390, 223), foreign cells (335, 336, 337) and foreign serum (109, 514). In acute adrenal insufficiency, the capacity to form antibodies is decreased (335, 337) although this may be attributable to loss of the medulla (336). The opsonic index of the adrenalectomized rat is reduced (26a). Cortical extract will restore the resistance to normal (184, 93). Desoxycorticosterone (93), unlike the corticosterones (235), is apparently ineffective against typhoid vaccine.

If the adrenal cortex is to be regarded as essential for the immunity reaction, cortical hormone should afford protection to the normal animal against infections and against toxins. Evidence on this point seems sharply divided. Protection against diphtheria toxin (527), anaphylactic shock (512), tuberculosis (346), and infections (240, 336a, 496) have been reported in intact animals and in humans. On the other hand, an effect against infections or toxins (392, 497), typhoid vaccine (142), or against anaphylactic shock (86) has also been denied.

3. *Changes in environmental temperature.* Either an appreciable increase (491) or a decrease (166, 169, 491, 90, 519, 200, 201) in the external temperature

will precipitate a fatal collapse in the adrenalectomized animal. The physiological reactions of the subject to cold have been carefully studied (200, 201) and a bioassay constructed on the basis of the results (405). The primary deficiency is an inability of the adrenalectomized animal to increase its basal heat production, attributed both to a general muscular asthenia and to a circulatory deficiency. The body temperature falls rapidly, and death follows. Unilaterally adrenalectomized animals also show a decreased tolerance to exposure to cold (201). Since adreno-cortical transplants offer complete protection, a deficiency of the adrenal medulla is apparently not involved (519). Restoration of the resistance to that of the normal animal can be obtained with cortical extract, and with all the cortical hormones, including desoxycorticosterone (405, 234, 523).

4. *Changes in environmental pressure.* The suggested relation of the adrenal cortex to the ability of an animal to become acclimatized to a reduction in barometric pressure is based on several points of evidence. First, the adrenalectomized animal shows little or no capacity for adaptation, so that moderate pressure reductions are fatal. The power of adaptation can be restored by cortical extract or by the corticosterones (253, 265, 456, 475). Second, the intact animal shows a marked hypertrophy of the adrenals after subjection to lowered pressure (456, 475). Third, adaptation is accompanied by a rise in liver glycogen levels and a rise in urinary nitrogen excretion. The increase in carbohydrate stores is in large part accounted for by the catabolism of endogenous protein (94, 253, 265). Fourth, the symptoms observed during the post-adaptive stage resemble in almost all respects those typical of adrenal insufficiency (456). The interpretation advanced is that the first subjection to lowered pressure increases the demand for the secretion of the adrenal cortex, so that a period of partial adrenal insufficiency follows, corrected only when the glands have become hyperfunctional. The eventual failure of the compensating mechanism possibly coincides with exhaustion of the adrenal cortex, and the development of an acute adrenal insufficiency.

In the light of this interpretation, it would be reasonable to assume that cortical extract would have a marked effect in allowing the normal animal to survive low pressures. Sundstroem and Michaels (456) give evidence in support of this suggestion, but the results of other workers are not so convincing. While cortical extract will correct the loss of sodium and chloride through the kidney (265) its effect in protecting life is not marked (265). Hence the evidence does not yet warrant the conclusion that the adrenal cortex is the sole or even a major factor concerned in adaptation to low pressures.

5. *Traumatic injuries.* The clinical picture and the blood chemistry changes shown by the animal in adrenal insufficiency are closely similar to those which characterize shock in the normal animal, suggesting that a common physiological deficiency might be involved (448). Loss of the adrenals does render the animal exceedingly sensitive to but minor amounts of trauma (437, 111, 451, 333, 332, 444, 490, 193, 402). Swingle and associates showed that none of the major features of adrenal insufficiency, such as hemoconcentration and internal fluid readjnstmeuts, changes in serum electrolyte concentrations, or the rise in blood

urea nitrogen levels, are essential characteristics of the shock-like circulatory collapse which follows trauma in the adrenalectomized dog (444, 355). A typical state of adrenal insufficiency has not, therefore, merely been precipitated by the experimental procedures.

It was shown earlier (449, 448), however, *a*, that the one most constant symptom of adrenal insufficiency in the dog was a slowly declining blood pressure; *b*, that the arterial pressure started to decline slowly but progressively from the time maintenance amounts of cortical extract were withdrawn; *c*, that the pressure could not be spontaneously raised to normal once it had fallen; and *d*, that the terminal collapse of the animal was always associated with extremely low blood pressure levels. The circulatory failure was attributed to a collapse of the peripheral circulation.

That the adrenal cortex is concerned in the ability of the normal animal to withstand traumatic injury would seem not unlikely, and has been postulated (193, 402). The proof would lie in the clear demonstration of a protective influence of cortical extract against the development of shock in the intact animal. To date such proof has not been forthcoming.

The use of cortical extract with saline or plasma transfusions was reported beneficial in treatment of toxemic shock following intestinal obstruction (511) and the injection of the contents of a closed intestinal loop (198). Fine and Fuchs and Mark (105) found that while desoxycorticosterone could prevent the decline in plasma volume after intestinal obstruction, it had no clear effect in preventing shock. Selye and associates (401,402) and Weil and co-workers (489) likewise reported that desoxycorticosterone was of no value in treating shock in rats and rabbits following intestinal trauma, but found that cortical extract or the corticosterones were useful. Helfrich, Cassels and Cole (195) observed that the blood pressure decline following intestinal manipulation was retarded by cortical extract therapy.

In the treatment of shock following hemorrhage, Helfrich and associates (195) reported a favorable effect of cortical extract, but this has been denied by both Fine, Fischmann and Frank (104) and Huizenga, Brofmann and Wiggers (206) in controlled experiments.

Katz and co-workers (229) and Shleser and Asher (409) presented rather striking evidence that both desoxycorticosterone and cortical extract could prevent shock in intact dogs subjected to an occlusion of the veins of one hind leg, but their results were not confirmed (436). Bourque and associates (27) found that while cortical extract was ineffective in preventing shock after venous occlusion, it did increase the efficacy of a salt infusion.

Noble and Collip (327) observed a slight protective action of cortical extract and desoxycorticosterone against shock induced by revolving rats in a specially designed drum. No evidence was found for a therapeutic value of either cortical extract or desoxycorticosterone against shock induced in the intact dog by leg muscle trauma, or following release of tight leg tourniquets, by Swingle and co-workers (436). Likewise, Ingle (210) observed no difference in the survival

of rats subjected to leg constriction by tourniquets after treatment with cortical extract, desoxycorticosterone or the corticosterones.

Perhaps the best evidence in favor of a beneficial effect of cortical hormones on shock is in the case of severe burns, where plasma electrolyte changes seem to be prevented (504), and the efficacy of a plasma transfusion is increased (358). While considerable work has been done on the use of cortical extract and desoxycorticosterone in treatment of shock in the human, the reports are conflicting, and the results difficult to assess (22, 222, 231, 245, 393, 504). It is apparent that in very few instances has the degree of protection reported against shock been of sufficient magnitude to warrant the conclusion that the stress procedure had induced circulatory failure by reason of a sudden partial adrenal insufficiency.

Selye (396, 397) has shown a close similarity between the symptoms of adrenal insufficiency and those exhibited by the intact animal when subjected to any of a wide variety of damaging agents, i.e., the alarm reaction. This, and other lines of evidence, indicates that a partial state of adrenal insufficiency may be involved. The basic mechanism involved is believed to be an inability to inactivate, in a normal manner, a histamine-like material released into the blood after subjection to the noxious stimulus. Both blood (503) and tissue (369, 370) histamine levels are elevated after adrenalectomy, presumably because histamine is inactivated less readily in the adrenalectomized animal (368).

In a study of the protection afforded by the various cortical steroids against circulatory failure in the adrenalectomized dog, Swingle and associates (435, 452, 243, 331, 355) found a correlation between the ability to prevent fatal circulatory collapse and the influence exerted upon carbohydrate metabolism. They concluded that the most likely interpretation is that the peripheral vascular system of the adrenalectomized animal had become weakened through a failure in intermediary metabolism, so that it was no longer able to maintain normal function in the face of a prolonged stimulation. Since there is no evidence of a primary failure of tissue metabolism in shocked intact dogs, it is not surprising to find that cortical hormones are without marked effect in increasing the normal resistance to shock.

On the other hand evidence has been presented that functional deficiencies of the heart, the arterioles and the capillaries may all be involved in the circulatory failure of the adrenalectomized animal suffering from adrenal insufficiency or shocked by traumatic procedures.

G. *Heart, arterioles and capillaries in adrenal insufficiency.* 1. *Heart.* Irregular heart rhythms are more or less frequent in acute adrenal insufficiency. Nicholson and Soffer (324) have suggested that they may be attributed to the rise in serum potassium concentrations. Cleghorn and associates (54, 55, 110) believe that a part of the circulatory collapse can be attributed to cardiac failure. Both cortical extract and desoxycorticosterone will correct the cardiac abnormality (55).

2. *Arterioles.* The evidence in support of an impairment of the functional

capacity of the arterioles in the adrenalectomized animal is entirely indirect. Hypertension cannot be produced in the adrenalectomized animals, even though salt is given (133, 25, 329, 63, 99). The sensitivity to injected renin is also lost shortly after the withdrawing of cortical extract (502, 115, 354), but can be restored by either cortical extract or desoxycorticosterone.

Elliott (89) first pointed out that splanchnic stimulation did not evoke a measurable rise in blood pressure in animals prostrate from adrenal insufficiency. Likewise, injected barium chloride and pitressin exert little effect (89, 10, 354). Adrenalin, however, produces a full pressor response (89, 10). This is true only for large doses of adrenalin, and comparable results are obtainable with large doses of renin (354). Coombs (64) and Langsdorf (254) presented evidence that there was a decreased sympathetic tone in adrenalectomized animals. Secker (395) believed that this loss in tone was due to a decreased production of the sympathetic chemical mediator, but the evidence upon which this conclusion was based has been contradicted (10, 190, 54). Fowler and Cleghorn (110) found, in acute adrenal insufficiency, no failure of splanchnic constriction and concluded that the absence of pressure rise is to be attributed to a failure of the heart to respond to the increased peripheral resistance. Whether cardiac failure can explain the decreased sensitivity to all pressor agents remains to be shown.

Wyman and tum Suden (518) found that removal of the adrenals changed the acute response to lethal doses of adrenalin from a rapid pressure fall, accompanied by lung edema, to a slow progressive circulatory failure, which might be taken as evidence for a failure in the capacity of vasoconstriction. Another line of evidence implicating the arterioles is derived from a study of the response of the adrenalectomized dog to a graded hemorrhage (355).

3. *Capillaries.* Considerable evidence, both direct and indirect, indicates that the capillaries of the adrenalectomized animal in insufficiency are atonic, dilated, and abnormally permeable. Such an explanation has been used to explain the fact that in adrenal insufficiency, and in experimentally induced rapid dehydration, the blood volume is reduced far lower than might be expected if there were a free transfer of fluid from the interstial reservoirs (352, 444). A serum transfusion will precipitate a circulatory collapse in the adrenalectomized dog, accompanied by an excessive edema (438). Either cortical extract or desoxycorticosterone prevents the edema formation and maintains life.

Menkin (302) showed that the leakage of trypan blue into extravascular tissues after the injection of leukotaxin could be reduced by either cortical extract or desoxycorticosterone. Fine and Fischmann (103) confirmed this finding for desoxycorticosterone, but do not believe a change in capillary permeability is necessarily involved. Freed and Linder (113) found that while cortical extract and corticosterone would prevent dye leakage after leukotaxin or peptone injections, desoxycorticosterone was without positive effect. Menkin (303) has criticized this work on the basis of the experimental technique employed. Shleser and Freed (410) found that cortical extract retarded capillary dye leakage after peptone injections, but the corticosterones were ineffective. Hechter and associates (192) found that desoxycorticosterone acted like the sex steroids in

increasing capillary permeability in the uterus, but observed no effect in other tissues of the body. Hechter (191) does not believe that an abnormal increase in the permeability of the capillaries of the adrenalectomized animal can explain the lack of resistance to histamine injections.

Cope and associates (65) observed the protein content of the leg lymph to be almost doubled after adrenalectomy, which they believe evidence for an increased capillary permeability. In later experiments Cope (66a) finds that following the injection of radioactive colloid into the blood stream the rise in concentration of radioactivity in peripheral lymph is markedly increased in dogs in adrenal insufficiency as compared to the same dogs in the normal state. After administration of cortical extract there is a change in capillary permeability permitting the retention of plasma protein within the blood stream. Levin and co-workers (261) and Hartman and associates (179) report that the long recognized increase in plasma protein in the adrenalectomized animals was due to an increase in the globulin fraction, while the plasma albumin, which is the more permeable fraction, was unchanged or even decreased. Levin (260) attributes this to a failure in albumin metabolism.

The adrenalectomized frog shows engorgement of the capillaries with stasis of the blood, which can be corrected in some degree with cortical extract (289, 459). Since life cannot be maintained with either cortical extract or salt in these forms, there is a question as to whether adrenal insufficiency in the frog is comparable to that of the higher forms. Zweifach and Chambers (524) found that cortical extract would prevent the capillary hyperemia which follows exposure, histamine injections, or manipulations with needles. Hymen and Chambers (207) also noted an effect in reducing the edema formation in the perfused frog leg.

H. *Adrenal cortex in lactation.* Considerable work, which cannot all be covered here, has been done on the relation of the adrenal cortex to lactation. In general, it is agreed that lactation is not induced in the hypophysectomized animal treated with lactogen unless cortical extract or the corticosterones are given (318, 322, 319, 320, 321, 135). Desoxycorticosterone is ineffective (322). Hartman and his colleagues believe that the adrenal cortex contains a specific lactation hormone, *cortilactin* (420). Others believe that lactation can be influenced by any agent which improves the general health of the adrenalectomized animal, such as sodium chloride (127, 321) and that normal lactation is induced with large amounts of cortical extract (127, 120, 322) or the corticosterones (120).

SUMMARY

Any attempt to prepare a concise summary of the rôle played by the adrenal cortex in physiological processes on the basis of existing evidence involves grave risk of oversimplification. It is tempting, however, to regard two general functions of the gland as pre-eminent, each regulated by a distinct type of steroid hormone elaborated by the cortex. The desoxycorticosterones, i.e., those lacking a keto or hydroxy group at Carbon 11 appear to be chiefly concerned with the regulation of electrolyte and fluid balance, acting 1, directly upon the renal

tubules to allow them to conserve sodium and water and to release potassium; 2, in a manner less clearly defined, on fluid and perhaps electrolyte partitioning across cell membranes, capillary endothelium and intestinal mucosa; and finally, 3, through their regulation of phases of mineral metabolism, primarily those involving sodium and potassium, to produce secondary effects upon extra- and intracellular hydration. Probably as a corollary of this last effect, the desoxycorticosterones allow the excretion in normal amounts of nitrogenous waste products and other metabolites.

The second established major function of the cortex is concerned with the intermediary metabolism of protein and carbohydrate. The oxygenated C_{21} steroids of the corticosterone type are those primarily involved in these phases of organic metabolism. A fault in the energy metabolism of the tissues of adrenalectomized animals lead to a (for lack of a more suitable term) general "asthenia", presumably, although this has not been demonstrated, of all the tissues, organs and organ systems, which is reflected in 1, failure of the work capacity of the skeletal muscle; 2, decrease in certain renal functions (e.g., elimination of excess water); 3, failure of lactation; 4, notably decreased ability of the vasculature to withstand even minor degrees of stress; 5, increased sensitivity of the organism to certain drugs and toxins.

It should be emphasized that all known facts of adrenal cortex function do not conform to this somewhat arbitrary division of functions of the desoxycorticosterones and corticosterones. The physiological activity of the two types of adrenal steroids overlap in many instances. The corticosterones are by no means entirely devoid of activity in so far as renal function and electrolyte metabolism are concerned, nor are the desoxycorticosterones entirely without effect upon organic metabolism and resistance to stress.

Interpretation of the functional significance of the desoxycorticosterones when contrasted with that of the corticosterones is rendered difficult by the fact that so much physiological data have been obtained with use of the synthetic desoxycorticosterone, which apparently is not elaborated in significant amounts in the gland itself, and which in large doses induces striking effects which cannot be produced by even excessive doses of the most potent natural extracts of the cortex. Among the twenty-odd steroids which have so far been isolated from the cortex, six are definitely known to be capable of maintaining life of adrenalectomized animals. Of the remainder a few may be weakly active, whereas most are devoid of known physiological function. However, it is not improbable that some members of this surprising array of steroid derivatives of the cortex may exhibit synergism when intermingled, as in whole gland extract or the natural secretion of the gland itself, and produce effects in adrenalectomized animals which the isolated steroids are incapable of inducing singly. Moreover, before the rôle of the adrenal cortex in physiological processes can be fully elucidated, much more needs to be known regarding the chemical nature and physiological activity of the amorphous fraction remaining after separation of the known cortical steroids from extract concentrates. While the amorphous fraction is known to exert striking effects upon certain aspects of renal function and to be

very potent in so far as life maintenance is concerned, little else is known concerning it.

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LIPOTROPIC FACTORS

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The term "lipotropic" was first used in 1935 by Best, Huntsman and Ridout (1) to describe the action of choline in the prevention and cure of fatty livers. For the purpose of this review a lipotropic factor is defined as a substance which prevents or removes an accumulation of excess fat in the liver. Three such factors will be considered: (I) choline, betaine and other related compounds, and choline precursors such as methionine; (II) lipocaic, the name used by Dragstedt to describe material extracted from pancreas; and (III) inositol.

I. CHOLINE, CHOLINE PRECURSORS AND RELATED COMPOUNDS. The discovery of the lipotropic action of choline was derived from studies on the production and prevention of fatty livers in depancreatized dogs maintained with insulin. In 1924 Fisher (2) reported the production of fatty livers in such animals and a similar observation was made simultaneously and independently by Allan, Bowie, Macleod and Robinson (3). Hershey (4), working in the laboratory of J. J. R. Macleod, reported that lecithin could be substituted for raw pancreas as a preventive measure against fatty livers in the diets of the dogs. More extended observations on the effects of lecithin were provided by Hershey and Soskin (5), and Best, Hershey and Huntsman (6) showed that choline could replace lecithin in the experimental diet.

It is interesting to consider the reason advanced by Hershey (4) for the attempt to use lecithin in places of pancreas. The latter had been incorporated in the diet of depancreatized dogs because it was assumed that the removal of the pancreas had caused a deficiency of digestive enzymes which would interfere with the absorption of foodstuffs. Hershey reasoned that the development of fatty livers did not result from any disturbance in digestion, but was caused by a derangement of fat metabolism in the liver. Reverting to the theory advanced by Leathes and Raper (7) that phospholipids were involved in fat transport from the liver, Hershey decided to feed a phospholipid to the depancreatized animals to supply an essential factor for fat metabolism and transport. This explanation was clearly given in Hershey's first report (4) and it is desirable to point out the soundness of his reasoning; the correctness of his hypothesis has been amply verified.

In a recent paper Engel (8) has stated that the first observation on the lipotropic action of choline was made by Junkersdorf and Kohl (9) some years previous to the work of Hershey. Careful reading of the paper published by these investigators has failed to show any reason for concluding that this is the first report on the lipotropic action of choline. Junkersdorf and Kohl studied, particularly, the effects of epinephrine and an epinephrine antagonist, choline, upon the amount of glycogen in the liver. Fat determinations were made but were meagre and inconclusive. The entire emphasis was upon the pharmacologic

effects of epinephrine and choline. The investigations of Hershey and the subsequent ones by Best and collaborators were approached from an entirely different viewpoint, one which is consonant with all subsequent work on the lipotropism of choline.

The production of fatty livers in depancreatized dogs requires a great deal of time and is expensive; the number of observations which may be made is limited. To overcome these difficulties, Best, Hershey and Huntsman (6) used normal rats in which fatty livers were produced by feeding a diet high in saturated fats. They found that the inclusion of lecithin in the diet prevented the accumulation of fat in the liver. In the light of more recent experiments it might be considered odd that fatty livers were actually secured in the early work. The basal diet, consisting of wheat, oats, corn meal, beef fat and bone-meal, had a choline content of about 100 mgm. per cent. Assuming that the animals ate 10 grams of this diet per day, they thus received 10 mgm. of choline, an amount sufficient to prevent fatty livers of the thiamin type. It would seem possible that the grain in this diet supplied an assortment of the B vitamins which would cause the production of a fatty liver somewhat resistant to the action of choline, but which could be prevented by large amounts of choline, supplied in lecithin, plus inositol, furnished by the grain.

Following the demonstrations that lecithin would prevent fatty livers in depancreatized dogs and in normal rats, Best, Hershey and Huntsman (10) studied the effects of components of lecithin. Sodium oleate, sodium glycerophosphate and ethanolamine were found to be without effect; choline was definitely lipotropic. It was also reported that betaine was active. The field was thus opened for the subsequent extensive investigations on choline and other lipotropic factors.

In the eight years following the initial paper on the lipotropism of choline Best and associates studied a number of aspects of the problem and similar work was begun in other laboratories. After collaborative work in Toronto, Channon began similar investigations in Liverpool. The principal developments will be summarized briefly and some of them considered in detail presently. As in the case of rats, mice were found to develop fatty livers which could be prevented by choline (Best, Huntsman and Solandt, 11). Because the basal diets originally used contained large amounts of choline, it was recognized that diets should be planned to contain as little choline as possible (Best and Channon, 12). The fatty livers produced by the ingestion of cholesterol were studied and it was found that large doses of choline were necessary to prevent such fatty livers (Best, Channon and Ridout, 13; Channon and Wilkinson, 14; Aylward, Channon and Wilkinson, 15; Best and Ridout, 16). Choline had no marked effect in preventing the accumulation of cholesteryl esters in the livers; it did prevent an accumulation of glycerides and, in curative experiments, caused a rapid decrease in glycerides and a slower diminution of cholesteryl esters. In the livers made fatty by feeding high-fat diets or in those caused by the inclusion of cholesterol in the diet, the principal effect of choline appeared to be on the glyceride fraction. Choline did not inhibit the deposition of fat in the livers when rats were poisoned

with phosphorus, but did accelerate the removal of fat from the liver during a recovery period; similar observations were made in rats treated with carbon tetrachloride (Best, MacLean and Ridout, 17; Barrett, Best and Ridout, 18; Barrett, Best, MacLean and Ridout, 19). While most of the results were obtained with high-fat diets it was noted that high-carbohydrate diets caused fatty livers when fed without choline (Best and Huntsman, 20). The deposition of liver fat was found to be affected by the amount of casein in the diet (Channon and Wilkinson, 21; Best, Huntsman and Ridout, 1; Beeston, Channon and Wilkinson, 22; Best, Grant and Ridout, 23). Other proteins, such as gelatin and defatted beef muscle, were found to be much less lipotropic. This protein effect was largely explained by Tucker and Eckstein (24), who showed that methionine was lipotropic and the action of methionine was made clear by the demonstration by du Vigneaud and associates (25) that this amino acid provided methyl groups for the synthesis of choline *in vivo*. Compounds related to choline were studied (Best and Huntsman, 26; Channon and Smith, 27). Betaine and triethylcholine, among others, were found to be lipotropic. Griffith and associates (28) contributed important observations on renal damage and on degenerative changes in other tissues, produced in rats fed a diet free of choline, or of choline precursors. Further information on the synthesis of choline, indicating the importance of ethanolamine, was provided by Stetten (29). The nature of the mechanism by which choline exerts its lipotropic effect was indicated by Welch (30) and by Perlman and Chaikoff (31). McHenry and associates (32), Engel (8), Forbes (33) and others have investigated the relation of the B vitamins to the production of fatty livers. Two other lipotropic factors, lipocaic and inositol, have been described. Developing from the initial work on diabetic animals there was, thus, a rapid extension of the investigations on lipotropic factors. The results have provided a new approach to the study of fat metabolism and have given new significance to several dietary constituents. A more detailed consideration of some aspects of the investigations will now be discussed.

1. *The action of choline on various types of fatty livers.* In the initial studies on the action of choline in rats, fatty livers were produced by feeding a diet rich in fat. The investigations were quickly broadened to include work on the effects of choline on fatty livers produced by feeding cholesterol, by toxic substances, and by other means.

a. "Cholesterol" fatty livers. Observations cited by several early workers (34–38) showed that the liver lipids can be changed in nature or in amount by the addition of cholesterol per se or of food substances rich in cholesterol to the diet of experimental animals. A more recent study was made in 1931 by Kimura (39) who noted that fat accumulated in the livers of rats which were fed cholesterol. Preliminary reports by Blatherwick and associates (40–42) presented evidence which showed that the ingestion of dried whole liver, raw liver, or cooked whole eggs caused fatty livers in rats. The fatty liver thus produced was resistant to the lipotropic action of lecithin. In later work, Blatherwick et al. (43) suggested that one substance, present in these dietary supplements and partially responsible for the observed deposition of fat, might be cholesterol,

although they felt that another factor was present. This explanation, in so far as cholesterol was concerned, was accepted by Beeston and Wilkinson (44). Okey (45, 46) in 1933 reported that when cholesterol was fed to white rats as 1 per cent of the diet there was a marked increase in liver fatty acids and in cholesteryl esters but not in free cholesterol. That the concentration of free cholesterol in the liver did increase if cholesterol feeding were continued for as long as three weeks was shown by Chanutin and Ludewig (47).

This type of fatty liver, characterized by a high content of cholesteryl esters, has been referred to by Channon and Best as the "cholesterol" fatty liver to distinguish it from the "fat" fatty liver produced by feeding high fat diets. It was reported by Best and Ridout (16, 48) that the cholesterol type of fatty liver could be partially but not completely prevented by a quantity of choline sufficient to control the fatty changes produced by the high fat diet alone. Excessively large quantities of choline did, however, exert a clearly marked influence on the liver fat. In a later paper Best, Channon and Ridout (13) stated that while choline will prevent the accumulation of neutral fat it has no effect on the free sterol and prevents only 60 per cent of the marked rise in cholesteryl ester which results from cholesterol feeding. Similar observations, which confirm these findings, were made in the same year by Channon and Wilkinson (14). The importance of the duration of the test period was indicated by the work of Best and Ridout (49), which showed that while choline caused no change in the cholesteryl esters during a twelve-day period it had caused a significant drop in the cholesteryl esters by the forty-first day.

Aylward, Channon and Wilkinson (15) found that the phospholipid content of the liver decreases for seven hours after a meal containing fat and cholesterol. This decrease could be prevented by the administration of choline. They suggested that either choline prevented the partial disappearance of liver phospholipid or its presence in the diet permitted the formation of new phospholipid to replace that which had disappeared. Further and more extensive observations, that the ingestion of cholesterol decreased the liver content of newly formed phospholipid even before the liver was fatty, and that choline stimulated the depressed phospholipid metabolism found under these conditions, were made in 1939 by Perlman and Chaikoff (50, 51). Several workers (21, 22, 52, 53) showed that protein exerted a lipotropic action on the glyceride fraction of the liver of rats fed cholesterol. It has been pointed out by Best and Channon (12) that the ease of removal and the degree of prevention of liver glyceride deposition in "cholesterol" fatty livers depends in part on the initial glyceride level. Best and Ridout (16) have observed that *a*, choline causes a rapid decrease in glyceride and a slower fall in cholesteryl esters, and *b*, that larger doses of choline are necessary to affect the cholesteryl esters than to reduce the glyceride fraction. They conclude, therefore, that the primary effect of choline is upon the glyceride fraction, although the possibility that choline affects both cholesteryl esters and glycerides directly cannot be eliminated in view of the fact that cholesteryl esters can be diminished while a large amount of glycerides is still present. Experiments by Channon and Wilkinson (14) and Loizides (54) have shown that the

cholesteryl ester content of the liver is increased by increasing the percentage of fat in the diet. The cholesterol fatty liver contains more glyceride than the "fat" fatty liver at any given level of dietary fat.

The work which has been reviewed above showed that choline, if given in large quantities, prevented an increase in simple glycerides in the livers of animals to which cholesterol was fed but had comparatively little effect upon the cholesterol content. Subsequent investigations have indicated that two other lipotropic agents, lipocaic and inositol, definitely prevent an increase in liver cholesterol. These will be discussed in later sections.

b. Fatty liver of starvation. During starvation, fat mobilized from the body depots accumulates in the liver (18). A paper on the pharmacological action of choline in starvation, by Junkersdorf and Kohl (9), indirectly called attention to an observation that the fatty infiltration of the liver which occurred on the eleventh day of starvation in dogs receded following choline administration. However, the data regarding fat contents were not sufficiently convincing as to warrant a conclusion that choline would prevent the fatty liver of starvation in dogs. Best (55) reported that choline administered to rats during a starvation period had little effect on the level of liver fat. However, the lipotropic activity of choline was clearly demonstrated if it was administered previous to and during the period of starvation. Best and Ridout (56) pointed out that the rat is not a suitable animal for the production of fatty livers by starvation since only adult female rats are so affected. Choline does not prevent the fatty liver caused by starvation in the mouse, rabbit or guinea pig. Deuel et al. (57) investigated the excretion of ketone bodies in the urine of rats previously fed a high fat diet with and without choline and the effect of choline administration to such animals during subsequent fasting. They found that choline added to a high fat diet controlled the liver fat (4.27 per cent) and that, during a subsequent period of fasting, liver fat increased (7.83 per cent). The administration of choline during fasting kept the fat at a minimum value and decreased ketonuria. It was concluded that choline does not increase the rate of fat oxidation, if ketonuria during fasting is an indication of such oxidation.

c. Fatty liver produced by toxic substances. The experimental data presented by Best, MacLean and Ridout (17) showed that choline inhibited neither the deposition of fat nor the production of degenerative changes in the livers of rats following the injection of large amounts of phosphorus. Choline did, however, increase the rate of disappearance of fat from the liver during the recovery phase. This latter observation was made also in 1936 by Laszt and Verzar (58). Large doses of choline prevented the accumulation of fat in the liver in rats poisoned with carbon tetrachloride; small doses were without effect (19). In investigations with carbon tetrachloride Barrett, Best and Ridout (18) showed by the use of deuterium that the increase of liver fat was due to a withdrawal of fat from the depots.

In connection with prevention of fatty livers by toxic agents it should be noted that Sato (59) reported in 1926 that the administration of an extract of liver lowered mortality in rabbits treated with chloroform. Subsequently Forbes,

Neale and Scherer (60) studied the preparation and value of a liver extract for the prevention of necrosis in the livers of rats poisoned with carbon tetrachloride vapor. The active constituent of the liver extract was claimed by Neale (61) to be xanthine. Confirmation of the results with liver extract and with xanthine was provided by Barrett, MacLean and McHenry (62). These workers demonstrated that xanthine protected the liver against necrosis and also accelerated healing following exposure of the animals to the toxic substances. Later, Forbes (63) pointed out that, while xanthine was effective, there appeared to be present in the particular liver fraction used by him an unidentified protective agent in addition to xanthine.

d. Fatty livers produced by anterior pituitary extract. In 1936 and in 1938 Best and associates (18, 64, 65) studied the effects of "ketogenic" fractions of anterior pituitary extracts in causing a marked increase in liver fat at the expense of body fat; it was found that choline did not prevent the fatty livers but hastened the removal of fat in the recovery phase. MacKay and Barnes (66) also reported that the fatty liver produced in rats by this means was not affected by the administration of choline, although choline reduced the accompanying ketonuria. The ineffectiveness of choline as a lipotropic substance under these conditions was confirmed in rabbits by Mukerji and Guha (67). Experiments reported by several workers in Dragstedt's laboratory (68) showed that lipocaic would prevent the fatty liver produced by the administration of anterior pituitary extract. This finding is contrary to that made previously by MacKay and Barnes (66). Dragstedt and his collaborators were of the opinion that the quantities of pancreatic extract used by MacKay and Barnes could not have been expected to exert definite activity. In view of the ineffectiveness of choline the reports regarding lipocaic are interesting and this effect of lipocaic deserves further investigation. This would be particularly valuable since the mechanism of production of fatty livers by anterior pituitary extracts resembles the processes involved in the production of fatty livers by starvation and by certain toxic agents. In all these cases the increase in liver fat is made possible by a withdrawal of lipids from depot stores. It would be most useful to ascertain whether lipocaic will prevent such withdrawals, especially since choline does not.

e. Dietary cirrhosis of the liver. Connor (69) considered fatty infiltration of the liver a prerequisite of liver cirrhosis and it has been noted by György and Goldblatt (70) that conditions which produce fatty livers in short term experiments lead to cirrhosis in experiments of longer duration. Similar deductions were made by Webster (71); Blumberg and McCollum (72) and by Sebrell et al. (73, 74).

In 1939 György and Goldblatt (75) reported that liver injury, in the form of acute diffuse necrosis combined with fat infiltration, could be produced somewhat irregularly in young rats on a diet composed of casein 18 per cent, sucrose 68 per cent, melted butter fat 8 per cent, cod liver oil 2 per cent, salt mixture 4 per cent supplemented with thiamin, riboflavin and pyridoxine. Diets containing more fat (50 and 70 per cent) and less protein (casein 10 per cent) had previously been shown to produce cirrhosis of the liver in a series of experiments by Blumberg

and associates (72, 76, 77). György and Goldblatt later (70) demonstrated that cirrhosis could be produced invariably when the casein content of the diet formerly used (75) was reduced to 10 per cent. These authors therefore considered that the dietary prevention of liver cirrhosis is related to the lipotropic action of casein. The incidence and severity of the liver injury were reduced but not completely prevented by a daily supplement of 10 to 20 mgm. choline per rat. Cystine, in doses of 10 to 50 mgm. daily, was shown to accentuate the cirrhosis while choline or yeast inhibited its production. The injurious effect of cystine on liver tissue had been reported by Curtis and Newburgh in 1927 (78) and at a later date by Earle and Victor (79). These authors (80) drew attention to the fact that the liver lesions were proportional to the level of cystine in the diet rather than to the total amount ingested and that choline prevented the effects of small doses of cystine but not of large ones. Choline in combination with cystine was reported by György and Goldblatt (70) to be more effective than choline alone in reducing the incidence and severity of liver damage. Methionine also prevented cirrhosis. This action of choline alone or in combination with methionine or cystine was mentioned in the report of Blumberg and McCollum describing experiments in which a high-fat diet low in choline and other lipotropic factors (72) was used.

Daft, Sebrell and Lillie (74) produced cirrhosis of the liver in rats when only a small quantity of cystine (0.5 per cent) was added to a diet low in both fat and protein. Choline, methionine and casein either singly or in combination prevented the development of cirrhosis. In a later paper (81) they showed that choline and methionine exert a preventive action on liver cirrhosis while cystine and methionine have a preventive action on hepatic hemorrhage and necrosis. This finding suggested to the authors that cirrhosis is an entity separate and distinct from hemorrhage and necrosis and there is thus offered an explanation for the observation made by the previous workers that choline and cystine in combination were more effective in reducing liver damage than either alone.

Fouts (82) has described recently the production in dogs of a syndrome consisting of anorexia, loss of weight, anemia, skin and peptic ulcers, and fatty cirrhotic livers. These conditions developed when the animals were maintained for some months on a low-protein ration with thiamin, nicotinic acid, riboflavin, pyridoxine and pantothenic acid. Provided that both pyridoxine and pantothenic acid were given, a high-protein diet appeared to protect the dogs. Fouts stated that small doses of choline (10 to 20 mgm. per kilo) were ineffective, that larger doses (100 mgm. per kilo) were partially effective, and that inositol exerted no beneficial effect. Partial improvement was secured with large amounts of choline, a powdered liver extract or a "filtrate factor" prepared from rice bran extract. After administration of large amounts of choline and powdered liver extract, deficiency signs cleared up rapidly, although hepatic fibrosis was still marked. The amount of inositol used by Fouts appeared to be too small to produce an effect and the same comment could be made about the dosage of choline. It would be interesting to try the effects of lipocaic in experiments of this kind.

This partial review of the literature on dietary production and prevention of liver cirrhosis in experimental animals indicates that this condition is caused by, or at least results from, the use of experimental diets which have been shown to cause fatty livers in rats. A diet low in protein, methionine, or choline favors the development of cirrhosis. Opinions have varied to some extent with regard to the value of choline but, on the whole, it seems to have been beneficial when given in adequate amounts. If the type of cirrhosis produced by dietary means in animals is the result of the maintenance of a high level of liver fat for an extended period, another useful approach could be made by the production of "biotin" or "cholesterol" fatty livers. While the results secured in animals have been valuable there has, as yet, been no proof that any cirrhosis in humans is a consequence of distortions of the diet.

2. *Relation of thiamin and other B vitamins to the production of fatty livers and to the action of choline.* In 1935 McHenry (83) reported that there appeared to be a complementary effect exerted by thiamin and choline upon the body weight of young rats. Animals fed a diet deficient in both choline and thiamin failed to increase in weight after an initial period of two weeks. Either choline or thiamin as a single supplement failed to give a sustained increase in weight but this was accomplished when the two supplements were given jointly. In a continuation of this work McHenry (84) found that rats would not develop acutely fatty livers on a low-choline diet unless thiamin was provided. At levels of fat intake from zero to 58 per cent of the diet thiamin had a marked effect on the amount of liver fat. This was true in experiments in which isocaloric feeding was used; the effect of thiamin was more extensive than simply an increase in appetite. Since the body fat was increased at the same time as was the liver fat the production of fatty livers was not due to withdrawal of fat from the depots. It was also stated that thiamin caused a marked increase in both liver and body fat in animals fed a fat-free, high-carbohydrate ration. In this case the vitamin seemed to promote synthesis of fat from carbohydrate, a conclusion which was in agreement with a report by Whipple and Church (85). The effect of thiamin in increasing fat by causing synthesis from carbohydrate can be appreciated but no adequate explanation has been offered of the action of the vitamin in producing fatty livers when a diet rich in fat was fed. Confirmation of this action of thiamin was furnished by Best and Ridout in 1938 (56).

It was also shown by McHenry (86) that the amounts of thiamin, choline and fat in the diet jointly affect the body weight of young rats. If the ration is deficient in thiamin, the inclusion of more than 26 per cent fat prevents a serious loss in weight. The effect of fat is augmented by adding choline to the diet and in this case an optimal effect is secured when the diet contains adequate amounts of choline and about 40 per cent fat. When thiamin is supplied without choline optimal weight increases are obtained when the dietary fat ranges between 10 and 26 per cent. The observations on the effect of fat when included in a thiamin-deficient diet are, of course, similar to those made previously by Evans and Lepkovsky (87) and confirmed in other laboratories since.

In a series of reports on the effects of the B vitamins upon fat synthesis,

McHenry and Gavin (88) found that the amount of fat synthesis is augmented by supplying other B vitamins with thiamin, although no synthesis was observed in the absence of thiamin. Riboflavin and rice polish concentrate were shown to have an augmentary effect. Halliday (89) reported that a deficiency of pyridoxine caused an increase in liver fat, an increase only partially controlled by choline but prevented by a liver extract used as a source of pyridoxine. Gavin and McHenry (90) reported that pyridoxine had no effect in lowering liver fat; these workers used pure pyridoxine and it is possible that the liver extract employed by Halliday contained large amounts of choline. In 1940 McHenry and Gavin (91) found that the administration of an extract of beef liver to rats, maintained on a fat-free diet, caused acutely fatty livers containing large amounts of cholesterol, provided that thiamin, riboflavin, pyridoxine and pantothenic acid were also supplied. Later they showed (92) that biotin, given in place of the liver fraction, had a similar effect and this type of fatty liver was referred to as the "biotin" fatty liver. This type is definitely resistant to the action of choline but can be prevented by lipocaic or by inositol. The "biotin" fatty liver is not, of course, the first one encountered in experimental work which has been shown to be resistant to choline. It can hardly be said that fatty livers produced by feeding cholesterol are readily susceptible to prevention by choline, nor are those fatty livers resulting from a withdrawal of fat from body depots, caused by starvation, by an anterior pituitary extract, or by a toxic agent. However, the observations on the "biotin" fatty liver were of value because they clearly indicated that all fatty livers produced by dietary means were not only dissimilar in genesis but also in response to lipotropic agents and probably in lipid composition as well. The effects of dietary constituents in altering the response of various fatty livers to lipotropic agents was further emphasized by the work of Engel (8) and of Gavin, Patterson and McHenry (93). In both cases it was shown that the administration of pantothenic acid and of pyridoxine altered the fatty livers so that they were somewhat resistant to the action of choline. A different result was obtained by Forbes (94); in this case nicotinic acid was found to increase the amount of cholesterol in the livers.

Observations on the effect of thiamin upon the production of fatty livers have been criticized by Griffith and Mulford (95) and by Handler and Bernheim (96). The first group was concerned, primarily, with effects of diet upon kidney lesions. To determine whether thiamin, and other B vitamins, had any influence upon the renal damage, the vitamins were added as supplements to a basal diet which contained 6 per cent yeast. It would hardly be expected that the extra supply of vitamins would have any effect, under such circumstances, and none was found. This is quite different from the results reported by McHenry, who studied the effects of thiamin deficiency. Griffith and Mulford found that a restriction in food intake to an amount about two-thirds of that consumed ad libitum protected rats from renal damage. From this result they drew the interpretation that thiamin influenced the production of fatty livers by increasing food consumption or growth. It should be made clear, however, that McHenry described experiments in which isocaloric feeding was employed and in which thiamin had a definite effect upon liver fat.

The experimental data of Handler and Bernheim are in substantial agreement with those of McHenry, and of McHenry and Gavin. Deficiencies of thiamin and of other B vitamins tended to prevent increases in liver fat of animals fed a choline-deficient diet. Handler and Bernheim studied the effects of various diets upon the rate of regeneration of liver tissue and upon the amount of liver fat in partially hepatectomized rats. Thiamin deficiency did not interfere with liver regeneration. To one group of 5 animals, which were choline-deficient, very large amounts of nicotinamide were given. As the authors had previously reported, the inclusion of 2 per cent nicotinamide in the diet caused a marked loss in body weight. The liver weights of unoperated rats and of partially hepatectomized rats were in fair agreement at the end of the experiment, but were not fatty despite the absence of choline from the diet. It was concluded that nicotinamide had not interfered with liver regeneration but if the data are examined it is clearly evident that the nicotinamide had so affected the liver as to cause a serious reduction in weight from that found in a comparable group without nicotinamide. Griffith and Mulford found that physiologically-sized doses of nicotinic acid had no appreciable affect upon liver fat and it may be suggested that the large doses of nicotinamide used by Handler and Bernheim had produced a toxic effect which had reduced the size and the fat content of the liver. Handler and Bernheim drew the following conclusion with regard to the effects of B vitamins: "It is suggested that the development of fatty livers in choline deficiency can proceed only when all other dietary factors will permit the growth of the whole rat rather than merely growth of the liver. The effect of deficiencies of members of the vitamin B complex in preventing the appearance of fatty livers due to choline deficiency is the result of an impairment of the over-all metabolism of the rat rather than some specific defect in the metabolism of the liver." Since this conclusion is based, largely, upon the claim of complete regeneration of the liver in partially hepatectomized rats fed large amounts of nicotinamide, and since the data do not support this inference, it seems wise to conclude that it has not yet been proven that thiamin deficiency does not cause a defect in liver metabolism. It may be said, however, that thiamin deficiency does inhibit the production of fatty livers and this is due to an alteration in those metabolic processes in which thiamin exerts an effect.

3. *Lipotropic effect of proteins and amino acids.* The first observation that protein influences the level of liver fat was indirectly made in 1935 by Best and Huntsman (20). In an attempt to repeat the work of Rosenfeld (97) on the curative effect of sucrose on fatty livers, rats in which fatty livers had developed were transferred to a diet of pure sucrose; an 8 per cent increase in liver fat resulted. This increase did not take place if the sucrose diet contained 20 per cent casein. At the time of this observation they were not certain whether this effect was due to the fraction of a milligram of choline which the diet provided or to a contaminant in the casein or whether casein provided, by its metabolism, betaines in effective amount.

While this work provided the first observation of the lipotropic activity of protein, Channon and Wilkinson (21), six months later, were the first to attach significance to the phenomenon. Experiments designed to study the effect of

various levels of dietary protein on liver fat showed that the degree of fat infiltration increased from 5.6 to 12.5 per cent as the casein content of the diet was decreased from 50 to 5 per cent. The suggestion was made that certain amino acids might be converted into choline or betaine in the tissues. Previous to this, Rosenfeld (98) had postulated that choline might be derived from glycine and as early as 1909 Engeland (99) proposed that betaines might arise in the tissues by the methylation of amino acids. In 1935, Best and Channon (12) further supported the supposition that some constituent of protein controls liver fat.

Beeston, Channon and Wilkinson (22) extended their work to include the influence of casein on the various fractions of liver fat. It was observed that an increase in dietary casein from 5 to 30 per cent resulted in a progressive decrease in the amount of liver glyceride and an increase in the amount of lecithin and cholesteryl oleate in the fatty liver produced by feeding cholesterol. These results suggested that the lipotropic effect of dietary protein should be differentiated from that of choline. In curative experiments Best and Ridout (53) reported that 50 per cent casein reduced cholesteryl esters to normal in rats which had received daily doses of 18 mgm. of cholesterol. In preventive experiments, Beeston et al. (22) were unable to demonstrate a lipotropic effect of casein on cholesteryl esters produced by the feeding of larger quantities (2 per cent of the diet) of cholesterol. The striking difference in the quantity of cholesterol used in these experiments may account for such conflicting results.

Casein was shown to exert a maximum lipotropic effect when it formed 30 per cent of a diet containing 40 per cent fat. One gram of casein was equivalent to 5 to 8 mgm. choline in its preventive action on liver fat (23, 100). The choline equivalent of casein was said to be greater in a fat diet than in a sucrose diet.

In an investigation of the effects of a number of amino acids, Beeston and Channon (101) added as little as 0.2 per cent cystine to a diet containing 5 per cent casein and 40 per cent fat and found that the quantity of liver fat was doubled. The anti-lipotropic effect of cystine could be offset by the inclusion of 30 per cent casein. This preliminary study failed to reveal an amino acid with lipotropic activity but it was shown that lysine, glutamic acid, aspartic acid, serine, glycine and phenylalanine had no effect either in increasing or in decreasing the fat in the liver. Beeston and Platt (102, 103) found that alanine, arginine aspartic acid, histidine, hydroxyproline, leucine, lysine, proline and valine were inactive; tyrosine showed slight lipotropic activity. Singal and Eckstein (104) confirmed the observation that dl-leucine and dl-valine were without effect on liver fat. They also reported that djenkolic acid and dl-isoleucine were lipotropically inactive.

In the year following the work of Beeston and Channon (101) on cystine, Tucker and Eckstein (24) confirmed the effect of cystine and were the first to report that methionine was lipotropic. In the light of this observation, it was suggested that the lower lipid values obtained by Channon and Wilkinson (21), when the diet contained 30 per cent casein, might be ascribed to the methionine content of the protein and that the two sulfur-containing amino acids exert opposite effects on liver fat.

The lipotropic effect of methionine was confirmed by Channon, Manifold and Platt (52) on both the glyceride and cholesterol fractions of liver fat. The lipotropic activity of methionine was calculated to be 1/12th that of choline, a figure which was later revised to 1/5th (105). Best and Ridout (106) also confirmed the lipotropic action of methionine and could find no difference between the activity of the d- and l-isomers in rats. Similar results were secured by Singal and Eckstein (107) in mice on low-protein, high-fat diets. The latter workers proved conclusively that cysteine and homocystine, like cystine, increase liver fat. They also showed that arachin, a protein of low methionine content, did not possess lipotropic activity. The low methionine content of gelatin may explain the lack of lipotropic effect of this protein, mentioned earlier by Best and Ridout (23).

Channon and collaborators (108) reported on the lipotropic activity of a series of proteins and listed them in the following order of decreasing intensity: gromax and whale muscle protein, caseinogen, albumin, beef muscle protein and edestin, fibrin and gliadin, gelatin and zein. In contrast to the results of Best and Ridout (23) they found that gelatin possessed slight but demonstrable lipotropic activity. In reference to this work Tucker and Eckstein (109) pointed out that, in general, the lipotropic activity of the above mentioned proteins paralleled their methionine content as determined by Baernstein (110).

The first suggestion that factors other than the methionine-cystine content of proteins may be involved in an explanation of the lipotropic activity of proteins was made in 1938 by Tucker and Eckstein (109). Contrary to expectation, they found that the addition of 0.5 per cent cystine to a diet of 5 per cent gliadin and 40 per cent fat failed to increase the liver fat values above that produced by the unsupplemented diet alone. A similar failure to demonstrate the cystine effect was observed by Channon et al. (105) when albumin was the source of protein in the basal diet. In view of the fact that both these proteins contain a high percentage of cystine, a more probable explanation may be that these diets provided more cystine than the amount (7 mgm./rat/day) shown by the work of Beeston and Channon (101) to cause a maximum deposition of liver fat. Channon et al. (108) found that albumin, which contains more methionine than does casein, exerts a slightly less lipotropic effect. In addition, they observed that various protein supplements behaved differently, depending on the nature of the basal protein. Best and Ridout (106) considered that their findings also favored the hypothesis that methionine could not be the sole lipotropically active agent in protein. They showed that the methionine and cystine content of a 30 per cent casein diet was not as lipotropic as the 30 per cent casein diet per se. Observations made by Tucker, Treadwell and Eckstein (111) were directly opposite. Best and Ridout also reported that a limiting lipotropic action of methionine was reached at a level of 0.5 per cent. This was confirmed by Channon, Manifold and Platt (105) who demonstrated that methionine, added as a supplement to an 8 per cent casein diet, exerted an increasing lipotropic effect up to 0.2 per cent, whereas the lipotropic effect of casein increased until it reached 30 per cent of the diet.

In the light of these findings Channon et al. (105) stated that "since methionine is probably not the only lipotropic constituent of caseinogen, two possibilities arise, either that some other amino acid also exerts a lipotropic action, or alternatively, added methionine is incapable of exerting its full action in the absence of some other protein constituent." They had no evidence bearing on the second possibility although they mentioned a suggestion made earlier by du Vigneaud (112) that the production of ethanolamine (possibly from an amino acid) might be the limiting factor. This was a most interesting speculation since Stetten (113) in the following year demonstrated that ethanolamine acts as a precursor in the biological synthesis of choline.

In view of the divergent findings of Best and Ridout, and Tucker and Eckstein, a reinvestigation of the comparative influence on liver fat values of casein diets and supplements of cystine and methionine in amounts equivalent to those present in casein diets was made by Treadwell, Groothuis and Eckstein in 1942 (114). This study confirmed their previous findings that diets supplemented with methionine were, so far as lipotropic action is concerned, superior to diets containing equivalent amounts of methionine in the form of casein. Treadwell et al. noted that the young rats on the high casein diet ate more food and gained weight more rapidly than rats on the methionine-cystine diet. They suggested that this would involve an increased demand for methionine in the formation of new tissue protein. The validity of this explanation could be determined by the use of the pair feeding technique.

The fact that liver fat values are affected by food intake has been demonstrated by Griffith and Mulford (95) in young rats. They explain the antilipotropic effect of cystine on the basis that 18 per cent casein diets contain an inadequate supply of dietary sulfur and that a supplement of 0.5 per cent cystine raises the metabolic level and thus creates an increased demand for lipotropic factors (115). On the other hand, the work of Treadwell et al. (114) indicates a direct antagonism between methionine and cystine.

Mention should here be made of the important work of du Vigneaud and associates who suggested (112) and later proved (25) that methionine exerts its lipotropic action, indirectly, by supplying methyl groups for the synthesis of choline. This work will be discussed in more detail in the following section.

4. *Biological synthesis of choline.* Since a very extensive review of the work on the bio-synthesis of choline has been presented by du Vigneaud (116) only the salient features will be noted here.

Du Vigneaud et al. (117) showed that homocystine could not support the growth of rats on a basal diet in which amino acids were used as the source of nitrogen and which was supplemented with thiamin chloride, riboflavin, nicotinic acid and ryzamin B. An equivalent amount of methionine was capable of supporting growth under the same experimental conditions. In contrast to the complete failure of growth with homocystine and the vitamin B mixture, the rats were able to grow when tikitiki extract and milk vitamin concentrate were added. It was concluded that there must be some factor present in the tikitiki and milk vitamin concentrate necessary for the methylation of homocystine.

The possibility that choline might be involved was suggested by the observation that fatty livers developed on the original diet but not on the diet supplemented with tikitiki and milk vitamin concentrate (112). Choline was therefore added to the original diet with homocystine. The animals gained weight at as rapid a rate as animals which had been given methionine in place of the choline and homocystine. The isolation of choline from the tikitiki and milk vitamin concentrate was further proof that it was, at least in part, responsible for the ability of those supplements to enable the rat to utilize homocystine in place of methionine. From these results it was inferred that a supply of methyl groups from choline made possible the conversion of homocystine to methionine.

Direct proof for the transfer of methyl groups from choline to methionine was demonstrated by du Vigneaud and collaborators (118) in a recent experiment in which deuterocholine and homocystine were fed to rats and deuteromethionine isolated from the tissue proteins. Thus the methyl groups of dietary choline are used in the bio-synthesis of methionine. Methyl transfer from choline to methionine was shown to occur even in the absence of dietary homocystine or in the presence of dietary methionine. The presence in the diet of a methyl acceptor is therefore not a requisite for transmethylation to methionine to take place. It was suggested that a precursor of methionine, undoubtedly homocysteine, is formed by the animal during the catabolism of methionine thus enabling methionine to be resynthesized with the methyl group supplied by choline.

Direct proof of the reverse transfer of the methyl group from methionine to provide for the formation of choline was later demonstrated by du Vigneaud et al. (25). Methionine containing deuterium in the methyl group was fed to rats on a methionine-choline free diet. The choline isolated from the tissues contained an amount of deuterium theoretically possible, if it was assumed that all the methyl groups of the choline had come from the deutero-methionine. The substrate that is methylated was shown by Stetten (113) to be ethanolamine, which is formed in the body by the reduction of glycine or as suggested by Folch (119) by the decarboxylation of serine. Glycine in turn can arise from the demethylation of betaine.

Proof that methionine also serves as a source of methyl groups for methylated compounds other than choline in the body and that the various N-methyl compounds synthesized by the animal derive their methyl groups from the "labile" methyl supply of the diet has been presented by du Vigneaud and co-workers (120) who found deuterium in the anserine isolated from rabbit muscle following the feeding of deuteromethionine.

Chandler and du Vigneaud (121) have shown that betaine will enable the rat to utilize homocystine in place of methionine although it is less effective than equivalent amounts of choline. The action of betaine in this regard is delayed for several days after its administration. The authors offer the explanation that choline is the compound which is actually used by the body for the methylation of homocystine but that betaine can be used after the organism acquires the ability to convert sufficient quantities to choline. The inequality of activity of

choline and betaine could be accounted for by a less than quantitative transformation of betaine to choline. That betaine is not directly converted to choline has been shown by Stetten (29). Betaine containing isotopic nitrogen was fed to rats and it was found that the glycine of the organ proteins and the phospholipid ethanolamine were rich in N_{15}. The phospholipid choline contained no more N_{15} than could be accounted for by the conversion of ethanolamine to choline. Therefore Stetten concluded that betaine is demethylated to form glycine, little or none of it being directly reduced to choline. It would appear that the lipotropic action of betaine is due eventually to synthesis of choline, which is not accomplished directly.

Stetten (122) has reported that the conversion of ethanolamine to choline proceeds without hindrance in rats even when the diet is sufficiently poor in labile methyl groups to cause fatty livers. Accordingly, the quantity of choline in the body is maintained at a constant level by the biological methylation of ethanolamine. This may explain the lack of relation of the choline content of the body to the amount of choline in the diet noted by several workers (13, 22, 123). Handler and Bernheim (124) reported that choline oxidase is greatly reduced or inhibited by the accumulation of fat in the liver and offer this as a possible explanation of the high content of body choline in the absence of available methyl groups. On the other hand, in confirmation of Engel's work (125), Stetten and Grail (126) have reported a drop in the choline content of the liver lipids when choline is omitted from the diet. They showed that in normal rats 20 to 30 per cent of the lipid nitrogen is choline nitrogen, whereas in animals on a choline deficient diet the corresponding figure is 11 per cent.

It has been clearly established that choline may be synthesized *in vivo* provided that methyl groups are available, as from methionine or betaine, and also that there is available a supply of ethanolamine. It would not be expected that symptoms of choline deficiency could be produced if the essentials for choline synthesis are supplied in the diet. While the validity of this has been established, there has been some confusion in some papers in the literature as to the rôle of methyl groups in fat metabolism. Realizing that choline can be synthesized and that the lipotropic action of choline is due to the stimulation of phospholipid formation, it seems unnecessary to assume, as has been done, that methyl groups play a direct part in the lipotropic action. Some discussion has taken place regarding the amount of choline present in the tissues of animals which have been maintained on a diet free of choline and of choline precursors. On such a regimen rats show a definite increase in liver fat within two days but it has been stated that the quantity of choline in the animal is not reduced. The most recent work (Engel, 125; Stetten and Grail, 126; Patterson and McHenry, 127) shows that the phospholipid concentration and the proportion of choline in the liver is decreased, as would be expected.

5. *Mode of action of choline.* The mode of action of choline as a lipotropic factor has yet to be determined conclusively. The most obvious explanation, and one that is supported by considerable experimental evidence, is that choline promotes lecithin synthesis in the liver. According to the early explanation of

Leathes and Raper (7), phospholipids are necessary for fat transport from the liver. The work of Bloor (128) and that of Sinclair (129) had definitely established the active rôle of phospholipids in fat transport. The widespread occurrence of choline-containing phospholipids and the relative constancy in amount with which they occur in tissues (130) has suggested that they are also concerned with cell structure. It has previously been pointed out that Hershey began the original investigation on the effects of lecithin in depancreatized dogs with a hypothesis derived from the explanation advanced by Leathes and Raper (7) regarding the transport function of phospholipids. In going back over the early literature regarding the lipotropic action of choline it seems strange that this simple concept of the rôle played by choline was disregarded in favor of more complicated and mysterious ones. In a space of a few years a series of hypotheses were advanced as, for example, that choline had an unknown effect upon liver function, or that it altered carbohydrate metabolism. Experimental evidence was not secured to substantiate these theories and the attention of investigators in this field was brought back to the obvious explanation which had formed the basis of Hershey's research.

Experimental evidence reported by Welch (30) and later by Stetten (29, 113) shows that dietary choline is used in the direct synthesis of phospholipid. In a clear-cut experiment Welch (30) fed rats arsenocholine, which he showed to be lipotropic, and found arsenic present in the lecithin of the tissues. Since the methyl groups of arsenocholine are not labile (131) Welch concluded that its lipotropic activity must depend on reactions which involve the intact molecule. It is reasonable to conclude that the lipotropic action of choline also involves the intact molecule rather than its labile methyl groups.

The methyl groups of choline do not appear to be necessary for its lipotropic activity since triethylcholine and arsenocholine both possess lipotropic activity as demonstrated by Welch but do not contribute to the labile methyl supply as shown by du Vigneaud et al. (112, 131). Mawson and Welch (132) have presented evidence which suggests that the hydroxyl group of choline is necessary for its lipotropic action.

Stetten (29, 113) fed choline, containing isotopic nitrogen, to rats and found that the choline of the body phospholipids was rapidly replaced by the ingested, labelled choline. The liver was reported to be the most active organ, with the other thoracic and abdominal organs next, closely followed by the carcass. The brain phospholipids are by far the least active with regard to choline turnover.

Perlman and Chaikoff (31) used radioactive phosphorus and showed that choline markedly stimulates the synthesis and transfer of phospholipid by the liver of the fat-fed rat. Choline caused a stimulation of phospholipid activity about one hour after its ingestion and its effect was still demonstrable for about 10 hours. It was observed that there was a relation between the amount of choline ingested and the phospholipid response within a range from 0 to 30 mgm. This observation is further evidence for the specific action of choline upon phospholipid metabolism.

Channon et al. (133) assumed that, if the lecithin hypothesis were correct, any

base having a choline-like action on liver fat should be incorporated in a new phospholipid molecule as a substitute for choline. Triethylcholine could not be detected in the liver phospholipid, following its administration to rats. While this negative result does not support the lecithin hypothesis, it should not be accepted without confirmation. On the other hand, Folch and Woolley (134) reported the presence of inositol, which is lipotropic, in certain tissue phospholipids. While significance has not been attached to this observation, it supports the theory of the synthesis of phospholipid in response to the administration of a lipotropic factor. Also in favor of this hypothesis is the observation made by Aylward, Channon and Wilkinson (15) that cholesterol, which is "anti-lipotropic", causes a fall in liver phospholipid. The fall in phospholipid occurred before the liver was fatty and it was checked by the administration of choline. They also noted that, in the absence of choline, as the phospholipid diminished, the glyceride increased. Welch and Welch (135) stated that the hypothesis that choline chloride is phosphorylated and utilized in the synthesis of lecithin is supported by the finding that the phosphoric acid ester of choline chloride is unaffected by liver phosphatase and therefore protected from oxidation in the liver.

Betaine, another lipotropic substance, accelerates phospholipid turnover. The magnitude of phospholipid activity was found to vary with the dose of betaine although the increase was not directly proportional to the amount fed (51).

Perlman and associates (136) extended their studies to include the effect of amino acids on phospholipid turnover in the liver and correlated this with lipotropic activity. Methionine was shown to produce an elevation in phospholipid turnover. Negative results were obtained with glycine, alanine, tyrosine, glutamic acid, asparagine, proline and serine. With the exception of tyrosine, which was reported by Beeston and Platt (102) to possess slight lipotropic activity, these findings agree very well with the inability of these amino acids to exert a lipotropic action as previously reported (101, 102, 103).

An exception to the general correlation between lipotropic activity and phospholipid turnover is the finding by Perlman et al. (136) that cystine and cysteine, while not possessing lipotropic activity, accelerate phospholipid turnover in the liver. Perlman et al., however, point out that the method of administering these amino acids differed from that in which their lipotropic action was studied. Observations of phospholipid turnover were made several hours after a single feeding while lipotropic activity was studied several weeks after daily feeding.

In the early work of Best and associates (13) on the effect of choline on liver fat it was reported that, while choline increases the percentage of phospholipids in the liver, its administration does not increase the proportion in which phospholipids containing choline occur in the phospholipid mixture. As Changus, Chaikoff and Ruben (137) have reported, the total phospholipid content of a tissue bears no relation to its phospholipid turnover. Brain, which contains twice as much phospholipid as liver or kidney, has but a fraction of the phospholipid turnover. It may be that a certain rate of phospholipid turnover must be maintained in order to prevent fat accumulation in the liver as well as other signs of choline deficiency.

Best (55) suggested that choline might affect liver fat by decreasing the amount absorbed or by increasing the oxidation of fat in the liver. Best and collaborators were unable to obtain experimental evidence to support either hypothesis. Deuel et al. (57) reported that choline does not increase the rate of fat oxidation, if ketonuria during fasting is accepted as evidence of such oxidation. MacLean, Ridout and Best (138) confirmed this conclusion by their observation that rats with fatty livers excrete larger amounts of ketone bodies during fasting than normal rats but the extent of ketonuria is not proportional to the excess fat in the liver.

It may be assumed that choline owes its lipotropic activity to its participation in the formation of phospholipids. From the many investigations on choline has come confirmatory evidence for the theory that phospholipids serve to transport fatty acids from the liver. For the formation of at least some of the phospholipids choline is required. This may be supplied in the diet or it may be synthesized *in vivo* if labile methyl groups and ethanolamine are available. In the absence of dietary choline or of choline precursors neutral fats accumulate in the liver. The principal result of the choline investigations has been to show that choline is necessary for the formation of some phospholipids and that fatty livers are produced if the mechanism for the transport of fatty acids is not available.

6. *Other effects of choline. a. Renal degeneration.* In 1939 Griffith and Wade (139) presented evidence for a hitherto unrecognized effect of choline deficiency, hemorrhagic degeneration of the kidneys. This deficiency was produced within 10 days in very young rats maintained on a low choline diet and was characterized by fatty liver accompanied by renal enlargement and marked hemorrhagic degeneration of the kidneys, a regression of the thymus and an enlargement of the spleen. Two milligrams of choline per day prevented the degenerative changes in the kidneys while 10 mgm. was required to control the liver fat. It was suggested (140) that choline has a more fundamental rôle than its function in the regulation of the fat content of the liver. The liver was fatty but the kidneys were normal if all the protein of the diet (15 per cent) consisted either of casein or of dried egg white. Hemorrhagic kidneys developed if 0.3 per cent cystine was added or if the protein consisted of 4 per cent fibrin, 8 per cent casein and 3 per cent dried egg white. The renal lesions could be prevented by the addition of choline or methionine. It was concluded (141) that the absolute amount of either methionine or cystine as well as the ratio of the two affects the choline requirement.

Previously Cox, Smythe and Fishback (142) reported the occurrence of hemorrhagic kidneys in rats on a casein diet to which cystine was added and Hartwell (143) noted similar lesions resulting from high edestin diets. The lesions could be prevented by increasing the supplement of marmite. Griffith's work (141, 144) suggests a correlation between these lesions and choline deficiency.

The histological changes in the kidney resulting from choline deficiency have been described by Christensen (145) and by György and Goldblatt (146) who pointed out their similarity to those resulting from cystine intoxication. Sup-

plements of cystine, fat or cholesterol, in the absence of choline, increase the severity of hemorrhagic degeneration of the kidney (147). The aggravating effect of dietary cholesterol is prevented during the crucial 8 day period but not during a subsequent 30 day period (95).

The age, weight and sex of rats affect the development of symptoms of choline deficiency (140, 148). It was found that renal lesions and fatty livers were most readily produced within 7 to 10 days in 40 gram male rats 20 to 30 days old. A marked decrease in the incidence of renal degeneration and liver fat content occurs in older and heavier rats. This suggests that there is a corresponding decrease in the choline requirement of older rats. Engel has reported (149) that symptoms of choline deficiency can be produced within 7 to 14 days at any time during the period of growth.

The choline requirement of the rat appears to be related, also, to the level of metabolism. It was noted (140, 144) that, if the dietary protein was adequate in amount and in composition for good growth, renal lesions and high levels of liver fat were produced more readily than if the protein was inadequate. Renal hemorrhage can be prevented by restricting the intake of a diet which when fed ad libitum causes the lesion (95). In the light of these observations and since the apparent injurious effect of cystine is not proportional to the level of added cystine, Griffith offered a tentative explanation of the injurious effect of cystine added to diets low in choline, methionine and cystine. On such diets there is insufficient methionine to satisfy the demand for choline and possibly for cystine also. A supplement of cystine, by improving the state of nutrition of the rat, increases the need for methionine and choline (150).

Since the studies of du Vigneaud et al. (25) showed that transmethylation occurs in the animal body and that methyl groups in such utilizable forms as methionine, choline and betaine may be essential in the diet, Griffith suggested that hemorrhagic degeneration of the kidneys might result from a deficiency of the indispensable labile methyl supply (150, 151). Methionine or betaine contribute to the labile methyl supply and may be substituted for choline in the prevention of hemorrhagic kidneys.

In animals which survive the crucial 10 day period of choline deficiency the kidney lesions and renal function show spontaneous improvement. The liver remains fatty throughout the period of recovery of the kidney; the recovered kidneys remain enlarged and in some cases are scarred with a white incrustation (148).

Engel and Salmon (152) used arachin, which contains very little methionine, as a source of protein in the diet of rats and observed fatal hemorrhages in the kidneys, eyes, adrenals and lungs. Weichselbaum (153) reported fatal liver hemorrhages in rats on diets deficient in cystine and methionine. These studies serve to confirm the importance of dietary choline for the maintenance of cellular nutrition.

Griffith (28) pointed out the probability that the choline phospholipids are involved in an explanation of renal hemorrhagic degeneration since lecithin is an intermediate in fat metabolism and important in cellular structure. He sug-

gested that "it is also possible that manifestations of choline deficiency, other than the fatty liver, may be due to the failure of formation of other essential methyl-containing metabolites." Previously Griffith and Mulford (151) had postulated that the renal degeneration was due to a lack of labile methyl groups, mainly because the feeding of methionine and betaine would prevent the lesions. It has been clearly established that the rat can synthesize choline from a supply of methyl groups in the diet. It would seem reasonable that methyl groups are needed merely for the synthesis of choline and not per se for the prevention of hemorrhagic kidneys since Welch found that triethylcholine (154) and arsenocholine (155), neither of which contribute to the labile methyl supply, prevent the development of hemorrhagic kidneys in rats on a low choline diet.

Observations made recently by Patterson and McHenry (127) have shown that renal damage, resulting from a low choline diet, is preceded by a decrease in the concentration of phospholipids in both the liver and kidney. Triethylcholine or choline prevent kidney damage and maintain the phospholipid concentration within normal levels. On the basis of these observations it was suggested that kidney lesions may result from a failure of a sufficiently rapid rate of phospholipid formation to maintain the structure of the growing kidney. While this hypothesis has yet to be proven it fits many of the known facts. Griffith (151) found that 1 to 2 mgm. choline would prevent the renal lesions, while 6 to 8 mgm. were necessary to reduce the liver fat. In accordance with the stimulating effect of choline on phospholipid turnover noted by Perlman and Chaikoff (31), it is conceivable that a small quantity of choline (1–2 mgm.) would stimulate turnover sufficiently to provide enough for the more vital cell structure while a large amount (6–8 mgm.) would be necessary to exert a demonstrable effect on liver fat. Griffith and Wade (140) have stated that renal hemorrhage can be produced most easily in only one brief portion of the rat's life, that immediately after weaning, and Engel (149) noted that it could be produced at any time during the period of rapid growth. During growth a supply of phospholipid may be particularly essential for the development of new cell structure. Changus, Chaikoff and Ruben (137) have shown that the rate of phospholipid turnover is much greater in younger rats than in older ones. Therefore, if choline is necessary to maintain phospholipid turnover, one could account for the requirement of choline being greater in young rats than in mature ones and also for the spontaneous recovery of the kidney at a time when the rate of phospholipid turnover is normally being reduced. Methionine, betaine and choline, which prevent renal damage, also stimulate phospholipid turnover. It should be noted that all substances which prevent this type of renal damage are lipotropic agents and it seems unnecessary to postulate any causative mechanism other than the rôle played by these substances in phospholipid formation and consequent fat transport.

b. Hyperplasia of the forestomach in rats. Hyperplasia and ulceration of the epithelium of the forestomach of rats fed bread and wood shavings was first observed in 1914 by Singer (156). Since that time several investigations have been made to determine the etiology of the lesion. While irritation by parasites (157) or by gastric juice (158) has been the only explanation advanced for the

direct production of the lesion, numerous reports have suggested that various dietary deficiencies are a predisposing factor (159, 160, 161, 162).

Dalldorf and Kellogg (163) and Sure and Thatcher (164) reported stomach lesions in rats suffering from thiamin deficiency. Similar lesions were obtained by Findlay (165) with a deficiency of vitamin B_2. Howes and Vivier (166) demonstrated that gastric lesions occur as a result of a deficiency of the vitamin B complex. This observation was confirmed by Sharpless (167, 168) who showed that lesions could be produced by a dietary deficiency either of protein or of the vitamin B complex.

Sharpless (168) demonstrated that the epithelial changes can be promoted in most rats fed a diet of white flour by providing supplements of riboflavin, nicotinic acid, cystine and an extract of rice polishings. In a later study Sharpless and Sabol (169) substituted thiamin, pyridoxine, choline and calcium pantothenate in various combinations in place of rice polish extract. When pyridoxine was added to the white flour diet containing thiamin and riboflavin, no protection was secured; choline protected all but 31 per cent of the animals; pyridoxine and choline fed together reduced the incidence of gastric lesions to 14 per cent; the further addition of calcium pantothenate had no additive effect. The results seemed to indicate that pyridoxine in the presence of choline aids in maintaining normal gastric epithelium. It was noted that animals fed the basal diet without choline had intestinal contents in the stomach. The stomachs of animals fed choline were free from visible intestinal contents. Sharpless and Sabol suggested that choline stimulated the smooth muscle of the gastro-intestinal tract and helped to prevent regurgitation of bile which might act as a stimulus to cell proliferation.

Sharpless (170) has reported additional data as a basis for an explanation of the gastric lesions. He found that sodium taurocholate or pepsin or hydrochloric acid increased the incidence of hyperplasia of the forestomach epithelium in animals fed diets deficient in cystine, riboflavin, pyridoxine or choline but not in animals fed a nutritionally adequate diet. The action of the protective factors is interdependent and a deficiency of one may prevent effective action by others. Sharpless postulated that "the mechanism of formation of gastric lesions is the irritation of abnormally sensitive epithelium by hair, hard food particles, pepsin and hydrochloric acid, or bile." The relation of choline to the prevention of these lesions is not clear.

c. Lactation. Sure (171) has reported that choline is an indispensable component of the diet for growth and lactation of the rat. When lactating rats were maintained on a diet deficient in choline the growth of the young suckling rats was arrested by the 13th day and paralysis and death ensued. The administration of 15 mgm. choline per day to the young rats or 5 mgm. choline to the mother rats and 10 mgm. to the young rats was required for satisfactory growth of the young. Choline was also shown to be necessary for growth of the weaned rat. The need for choline by the young rat is probably not a manifestation of a new function of choline but is related to the requirement of choline for normal phospholipid formation which is intimately concerned with fat transfer. Suckling

rats are receiving a high fat diet, since maternal milk contains 32 per cent fat (171). The phospholipid turnover is higher in the suckling rat than during any period of growth and consequently there would be a greater demand for choline during this period (172, 173). Artom and Fishman (174) have recently shown that choline administration is necessary to maintain the normal concentration of choline phospholipids in the liver of the newly weaned rat, maintained on a diet containing small amounts of choline or of choline precursors.

d. Perosis. Perosis or "slipped tendon" has been extensively studied in the chick. Two excellent reviews of the studies on the preventive effect of manganese on this syndrome have been presented by Jukes (175) and by Wilgus et al. (176). In this review reference will be made only to the relation of choline to perosis. In 1939 Jukes (177) observed that turkeys were highly susceptible to perosis and that it was not prevented in this species by the inclusion of manganese in the diet. In the following year Jukes (178, 179) described experiments which showed that choline, in addition to manganese, was effective in preventing perosis in both turkeys and chicks. It was noted that an adequate supply of the vitamin B complex was necessary for the full anti-perotic effect of choline to be exerted and that arsenocholine was anti-perotic while gelatin was completely ineffective. In contrast to the results secured with turkeys, Jukes (180) observed that chicks did not develop perosis on a choline deficient diet unless gelatin or creatine were added. Choline at a level of 0.1 per cent prevented perosis under these conditions. This effect of creatine was explained on the basis of the observation made by Almquist and Mecchi (181) that creatine deficiency results in muscular dystrophy and, if the tension on the bones is reduced by muscular dystrophy, the tendency toward perosis may be lessened. Glycine, the principal amino acid in gelatin, is a precursor of creatine. A study by Jukes and Welch (182) of the effect of certain analogues of choline on growth and on perosis in chicks showed that the anti-perotic activity of choline is distinct from its growth-promoting action. That fatty livers are not concerned in perosis resulting from choline deficiency was observed by Hegsted, Mills, Elvehjem and Hart (183), who confirmed the need for choline in the diet of the chick and noted that chicks suffering from perosis due to choline deficiency at 4 weeks of age did not show fatty livers. Hogan et al. (184) agree that choline definitely has perosis-preventing activity but their investigation indicated that choline was not the only specific nutrient present in such natural anti-perosis supplements as liver. No adequate explanation has been provided regarding the function of choline in the prevention of perosis.

(*e*) *Acetylcholine formation.* A preliminary experiment made by Solandt (185) suggested that a dietary deficiency of choline may result in a deficient formation of the neurohumour, acetylcholine. This hypothesis was tested in later experiments (186) in which it was observed that vagus stimulation reduced the heart rate to approximately 30 per cent of the normal value in rats on a normal diet; to 45 per cent in rats on a low choline, plus choline, diet; to 75 per cent in animals on a low choline diet. Intravenous injections of choline chloride in the latter group increased the action of the vagus on the heart muscle. This

phenomenon was never observed in rats in either of the groups receiving choline regularly. Solandt and Best concluded that a low intake of dietary choline causes deficient vagus function which can be rectified by injected choline. It was suggested that choline deficiency results in deficient formation of acetylcholine at the nerve endings. No more definite connection between choline in the diet and the liberation of acetylcholine at the nerve endings has so far been reported. These observations are of interest because they indicate that dietary choline may serve in a capacity quite different from that which explains the lipotropic function.

(*f*) *Butter yellow, hepatic tumors and choline.* The effect of various dietary factors on the hepatic tumors produced by adding butter yellow to a diet of unpolished rice and a small amount of carrot have been investigated by numerous workers.

The incidence of tumors in rats was markedly reduced by rice-bran oil (187), yeast (188, 189), dried beef liver (190) or the replacement of rice by wheat bread (188, 191). Sugiura and Rhoads (192, 193) found that an extract of rice-bran or an ether extract of yeast caused a transitory inhibition of liver cancer.

Kensler, Sugiura and Rhoads (194) reported that the contents of coenzyme I and of riboflavin in the livers of rats on the basal rice diet plus butter yellow were markedly reduced below normal values. The addition of 15 per cent whole yeast increased the levels to normal. In the following year these workers (195) studied the effect of nicotinic acid, riboflavin, and casein on the susceptibility of rats to cancer. These three supplements administered separately gave no protection but the administration of nicotinic acid with riboflavin resulted in a definite decrease in cancer incidence, while casein plus riboflavin gave striking though not absolute protection. They concluded that at least two factors, riboflavin and casein, render the rat less susceptible to the carcinogenic effect of butter yellow. György, Poling and Goldblatt (196) confirmed the beneficial effect of casein and noted that the combined administration of cystine plus choline afforded definite but not regular protection against the pathological changes in the livers produced by butter yellow. Du Vigneaud et al. (197) found biotin to be procarcinogenic when administered to rats receiving butter yellow and an otherwise highly protective diet.

Miller et al. (198) studied the effects of various modifications in the basal ration on the rate of tumor formation with butter yellow, particular emphasis being placed on the relative effectiveness of various proteins. Dietary casein (18–40 per cent) offered partial protection; liver, yeast and egg gave equivalent protection at levels of 12 to 13 per cent of total protein; 10 per cent whole dried beef liver in a 10 per cent casein diet or at a 20 per cent level as the sole source of protein offered nearly complete protection at 4 to 6 months; 1.2 to 2 per cent water-soluble, alcohol-insoluble portion of whole liver in a 10 to 12 per cent casein diet gave nearly complete protection at 4 months but not at 6 months; xanthine, l-cystine, i-inositol, and choline (0.1–0.5 per cent levels) gave no protection. Feeding experiments indicated that the dye reduced growth per se as well as by causing a decreased food intake. The authors concluded "(a) that nutritionally adequate diets offered at least partial protection against hepatoma formation

(b) that protective supplements were usually rich in both protein and the vitamin B complex, particularly riboflavin (c) that non-protective diets were deficient in at least one of these factors."

Jacobi and Baumann (199) demonstrated that butter yellow contains labile methyl groups and suggested that it may cause tumor formation by stimulating phospholipid turnover which is higher in tumor tissue than in normal tissue. Choline was found to be without effect on tumor growth. However, the tumor tissue may draw on the host for its supply of choline or it may itself synthesize this substance.

(7) *Is Choline a Vitamin?* There has been some debate as to whether choline should be classified as a vitamin, and more particularly as a member of the vitamin B complex. It is difficult to see why choline should be called a vitamin. The word vitamin was originally used to describe an unidentified substance, believed to be an amine, present in foods in minute amounts, and necessary for life. The chemical constitution of choline was known before any dietary significance was attached to it. No useful scientific purpose would be served by considering choline to be a vitamin. The attempt to call choline a B vitamin is even less excusable. There is evidence, either conclusive or suggestive, to warrant the opinion that the B vitamins are components of enzyme systems. There is no evidence that choline acts in this capacity. The only function of choline for which there is definite evidence appears to be that it is a structural constituent, either in the formation of phospholipids or for a supply of methyl groups. It would appear to be more satisfactory to leave choline unclassified, other than to say that, under certain conditions, it is an essential constituent of the diet. This is particularly true in view of the commercial exploitation of vitamins. The abortive attempt to call an essential fatty acid a vitamin is familiar to everyone, as is the effort to use this classification for commercial purposes. The reviewers can see no advantage, and several disadvantages, in labelling choline as a vitamin. It should, indeed, be remembered that choline only becomes a dietary requisite when the supplies of methionine and ethanolamine are markedly restricted.

(8) *Lipotropic Activity of Compounds Related to Choline.* Experiments described by Best and Huntsman (26) to study the effect of various components of lecithin on the fatty liver of the rat showed that betaine has a lipotropic action. Platt (200) reported that the activity of betaine, in this regard, was about 30 per cent that of choline. In an experiment which indicated that the hydroxyl group of choline is necessary for its lipotropic action, Mawson and Welch (132) reported that trimethylammonium chloride caused an increase in liver fat, while trimethylethyl, tetramethyl and trimethylphenylammonium chloride were so toxic that measurements of lipotropic activity could not be made. According to Channon and Smith (27) triethyl β hydroxyethylammonium hydroxide possessed two-thirds the lipotropic activity of choline weight for weight. Tripropyl β hydroxylethylammonium hydroxide had little, if any, lipotropic effect while homocholine (trimethyl γ hydroxypropylammonium hydroxide) was more effective than choline itself on both the fat-fatty liver and the cholesterol-fatty liver

(201). Observations by Welch and Welch (135) indicated that N-betaine aldehydechloride, and N-betaine hydrochloride, and the phosphorus and arsenic analogues of choline chloride were more than one-half as active as was choline chloride. Phosphorus and arsenic betaine hydrochloride were not lipotropic. This suggests that the phosphorus and arsenic analogues of choline act per se, or as corresponding aldehydes. Platt (200) has reported that etherification of the alcohol group of choline to form choline methyl ether destroys its lipotropic effect; tetra β hydroxyethylammonium chloride has no lipotropic activity.

TABLE 1

Lipotropic activity of compounds related to choline

COMPOUND	LIPOTROPIC ACTIVITY	REFERENCE
Arsenobetaine hydrochloride	−	135
Arsenocholine chloride	+	135, 30
Betaine	+	26, 135
Betaine aldehyde chloride	+	135
Betaine aldehyde acetyl chloride	+	135
Calcium phosphorylcholine chloride	+	135
Choline chloride	+	26, 10, 135
Choline methyl ether	−	200
Diethylmethylhydroxyethylammonium chloride	+	Cited in 131
Dimethylethylhydroxyethylammonium chloride	+	131
Homocholine	+	201
α methylbetaine hydrochloride	+	135
β methylcholine chloride	−	135
β methylcholine ethyl ether	−	135
α methyl-β-phenylcholine chloride	?	135
Phosphobetaine hydrochloride	−	135
Phosphocholine chloride	+	135
Tetra-β-hydroxyethylammonium chloride	−	200
Tetramethylammonium chloride	toxic	132
Triethyl-β-hydroxyethylammonium hydroxide	+	27, 133
Trimethylamine oxide hydrochloride	−	135
Trimethylammonium chloride	−	132
Trimethylethylammonium chloride	toxic	132
Trimethylphenylammonium chloride	toxic	132
Tripropyl-β-hydroxyethylammonium chloride	−	201

A summary of the qualitative lipotropic activity of various compounds related to choline is given in table 1.

From a consideration of the various compounds which have been shown to be directly lipotropic it would appear that the general configuration of the choline molecule is essential but that some particular segments are not. Methyl groups may be replaced by ethyl, as in triethylcholine. Nitrogen may be replaced by other elements with suitable valency (arsenic, phosphorus). A free hydroxyl group does seem to be necessary. These statements apply to compounds which, like choline, directly participate in the formation of phospholipids but not, of

course, to those substances which exert a lipotropic effect indirectly because they supply groupings which are required for the synthesis of choline, or of a choline-like compound.

(9) *Distribution of Choline.* Several methods have been used for the estimation of choline. It is widely distributed in animal tissues, as would be expected in view of the occurrence of lecithin. It has not been realized by some investigators that many substances used in the preparation of experimental diets are excellent sources of choline. This is particularly true of tissue extracts which have been employed to supply unidentified vitamins. It has seemed useful to summarize available information regarding the occurrence of choline in substances used in animal diets, mainly for the purpose of emphasizing precautions which must be taken in investigations in which effects of choline are to be studied. Practically all of such information is contained in two papers, one by Fletcher, Solandt and Best (202), the other by Engel (203). Table 2 gives the average values recorded by these workers.

A number of instances are available in the literature to demonstrate the need for care in planning animal dietaries. In the initial investigations by Best and associates (10) a grain diet was employed; this contained such large amounts of choline that it is difficult to understand how fatty livers were secured. A more recent example is contained in a paper by Quackenbush et al. (204). These workers noted that the addition of thiamin to the diet failed to cause fatty livers in rats. The diet contained 8 per cent autoclaved yeast, which would have supplied sufficient choline to prevent fatty livers. If the effects of choline are to be studied, or alterations in fat transport noted, not only must consideration be given to the actual choline content of the basal diet but in addition the presence of choline precursors must be taken into account. Choline deficiency cannot be expected unless attention is given to all dietary sources of choline and of those substances which make possible the *in vivo* synthesis of choline.

II. Lipocaic. After the initial work by Hershey (4) the assumption could have been derived from the literature that the value of the inclusion of pancreas in the diet of depancreatized dogs was due to its content of lecithin or choline. A modification of this assumption was introduced in 1935 by the demonstration of the lipotropic effect of protein. Investigations by Ralli, Flaum and Banta (205) in the same year suggested that raw pancreas was more effective in controlling the liver fat of depancreatized dogs than could be accounted for by its content of lecithin.

In 1936 Dragstedt and associates began investigations on the control of liver fat in depancreatized animals. In their first reports (206, 207) they concluded that the development of fatty livers is not due to the absence of pancreatic juice and that pancreatic tissue contains a lipotropic factor other than choline. The authors reported that at least 1.0 gram of choline was necessary per day to prevent fatty livers, while an effective amount of pancreas contained about 60 mgm. of choline. In view of later developments it should be pointed out that Dragstedt et al. tried liver or brain in place of pancreas; not only were these without effect but it might be concluded from the paper that the inclusion of liver in the diet had

accelerated the fatty infiltration of the liver of the experimental animals. In extending these observations Dragstedt, Prohaska and Harms described the preparation of an active extract from pancreas. The name "lipocaic" was suggested for the component which prevented the development of fatty livers. Because it was believed that pancreas alone contained lipocaic it was claimed to be a new hormone concerned with the normal transport and utilization of fat.

Significant contributions to the study of the effects of pancreas and choline were made by Kaplan and Chaikoff (208, 209, 210). Unfortunately, one of these observations was lost sight of by subsequent workers and has not yet received adequate attention. These workers showed that depancreatized dogs maintained with insulin showed a decrease in blood lipids as well as an increase in liver fat. They found that the feeding of raw pancreas prevented the increase in hepatic fat and raised the blood lipids above normal. This dual effect was not obtained with choline, or with choline plus autoclaved pancreas. They concluded that

TABLE 2

Choline content of substances used in animal diets

(Milligrams per 100 grams)

SUBSTANCE	FLETCHER	ENGEL	SUBSTANCE	FLETCHER	ENGEL
Beef liver	270		Vitamin free casein	4	
Beef pancreas	230		Agar	1	
White flour	140		Crisco	0.5	
Polished rice	94		Potato starch	0	
Skim milk powder	90		Cane sugar	0	
Rice flour	69		Mazola (corn oil)	0	
Bone meal	30		Dried baker's yeast	270	460
Corn starch	25		Dried brewer's yeast	240	430
Dried egg white	18		Peanut meal		225
Rice starch	10		Soy bean meal		345

pancreatic tissue contains two factors active in lipid metabolism: 1, a heat-labile factor which controls blood lipid levels, and 2, a heat-stable substance which prevents fat deposition in the liver. The existence of these two particular factors was never subsequently confirmed and their existence might be doubted. However, it seems likely that the heat-stable factor was choline, or perhaps choline plus another substance. The most significant conclusion derived by Kaplan and Chaikoff, and one which was not considered by most subsequent investigators, was, "although choline influences the fatty livers of rats fed a high-fat, low-protein diet as well as those of depancreatized dogs maintained on a low-fat, high-protein diet, the evidence available at present does not permit the conclusion that the mechanism of production and cure of these two types of fatty livers is the same. Hence conclusions derived from experiments on rats cannot be applied at present to depancreatized dogs." If this concept, that all fatty livers are not identical, had been taken into account, some of the subsequent confusion regarding lipocaic would not have appeared.

Shortly after the publication of the initial papers by Dragstedt and associates, the lipotropic effect of lipocaic was examined on rats in several laboratories. Overlooking the precautionary conclusion of Kaplan and Chaikoff, all of these investigations except one involved the use of a type of fatty liver which had been shown to respond readily to choline. Best and Ridout (211) found that lipocaic had no activity in rats other than that which could be accounted for on the basis of its content of choline and protein. While they carefully pointed out that lipocaic might contain a factor affecting the deposition of fat in the liver of the depancreatized dog but not in the "normal" rat, the inference could have been drawn easily that lipocaic had no special effect. Similar conclusions were expressed by Aylward and Holt (212) and by MacKay and Barnes (213). All three investigations were carried out with rats fed a low-protein, high-fat diet. In contrast with these negative findings, Channon, Loach and Tristram (214) reported evidence showing that pancreatic extracts contain a lipotropic agent distinct from choline or protein. The positive results were mostly secured in rats which had livers made fatty by feeding cholesterol. Unfortunately, in the light of later work, no observations were made upon liver or blood cholesterol values. Channon and associates, like Kaplan and Chaikoff, attempted to distinguish between the conventional type of dietary fatty livers in rats and those found in depancreatized dogs. They pointed out that depancreatized dogs develop fatty livers on protein-rich diets which would inhibit the production of fatty livers in rats.

In connection with an investigation of the causes and prevention of fatty livers in depancreatized dogs, Ralli, Rubin and Present (215) noted in 1938 that the livers showed a marked increase in cholesteryl esters but no particular change in free cholesterol. In the following year, Dragstedt et al. (216) described blood lipid changes co-incident with the development of fatty livers: a reduction in total lipids, and of cholesteryl esters. The administration of lipocaic caused an increase in blood lipids.

Dragstedt (217) reviewed the status of lipocaic in 1940 and it is advantageous to consider some of the features of the review. He stated that the depancreatized dog suffers from two obvious deficiencies: insulin and pancreatic juice. Adequate dosage with insulin does not prevent the production of fatty liver, nor does a supply of pancreatic juice (see next paragraph). The feeding of raw pancreas is completely effective as a lipotropic agent as also are highly purified pancreatic extracts containing entirely ineffective amounts of choline. Under conditions used in Dragstedt's laboratory, the minimal effective dose of choline was found to be 2 grams per day. In the review Dragstedt emphasized his belief that lipocaic was an internal secretion of the pancreas. If this supposition is granted, the pancreas supplies two hormones: insulin and lipocaic. After removal of the pancreas the deficiency of insulin is manifest within a few hours, as is the case with other hormones, following gland extirpation. Fatty livers do not develop for a period of some weeks, or perhaps months. According to Dragstedt, these fatty livers are caused by a deficiency of lipocaic. How can the very slow development of fatty livers be explained if lipocaic is a hormone? It seems un-

likely that a long interval of time would be required for the accumulation of fat in the liver, if suitable conditions existed. It would seem more plausible that there is a storage of lipocaic in the body and that fatty livers develop when this store is exhausted. The process of fatty liver formation in the depancreatized dog is more similar to the development of a dietary deficiency than it is to the consequences of the absence of a hormone.

The conclusion of Dragstedt and associates that the administration of pancreatic juice to depancreatized dogs will not prevent fatty livers has been challenged by Ralli, Rubin and Present and by Montgomery, Entenman and Chaikoff. Ralli et al. (215) reported that ligation of the pancreatic ducts causes fatty livers which are indistinguishable from those seen in depancreatized animals. In extensive investigations Montgomery, Chaikoff and others (218–221) have secured a similar result and have found that the feeding of pancreatic juice is as beneficial as raw pancreas. These workers showed that the fatty livers of depancreatized dogs definitely contain large amounts of cholesterol.

A further factor in the production and prevention of fatty livers in depancreatized dogs was introduced by Ralli and Rubin (222). Such dogs are customarily fed large amounts of meat. Ralli and Rubin claimed that, if the meat was extracted with alcohol, fatty livers were not produced. The addition of the extractive to a diet containing extracted meat led to the formation of fatty livers. Previous observations on the formation of fatty livers in depancreatized dogs all pointed to the cause being a deficiency (of pancreatic juice, or of lipocaic, or of choline). These observations of Ralli and Rubin suggested the existence of a positive lipogenic factor, ordinarily present in the diet.

In 1931 Blatherwick and associates (40–43) began the publication of a series of papers dealing with the production of fatty livers in rats by feeding liver. If the liver was previously extracted with alcohol or water it was ineffective. Blatherwick et al. concluded that liver contains a factor which causes fatty livers, although they did not carry out a positive experiment in which extractives from liver were administered. The resultant livers contained large amounts of cholesterol and, as the authors pointed out, this might have been due to the high content of cholesterol in the liver included in the diet. The production of the fatty livers was not prevented by supplying sufficient lecithin to furnish 143 mgm. of choline per rat per day. Beeston and Wilkinson (44) were unable to confirm the observations of Blatherwick et al. and believed that the effects of the inclusion of liver in the diet were due entirely to the cholesterol thus contributed. Much the same conclusion was advanced by Best, Channon and Ridout (13), who also explained the lack of effect of lecithin as being due to inadequate dosage.

In connection with an investigation on the effects of various B vitamins upon fat synthesis and metabolism McHenry and Gavin (223) tried the effect of a 90 per cent alcohol extract of liver, used as a source of unisolated vitamins. This extract was given to rats as a supplement to a high-carbohydrate, fat-free diet. Within 7 days the animals developed acutely fatty livers containing large amounts of cholesterol. The basal diet and the supplements contained negligible amounts of cholesterol and the results clearly confirmed the finding of Blatherwick and as-

sociates that liver contains a factor causing fatty livers. The fatty livers produced by the liver extract were highly resistant to the action of choline but were easily prevented by lipocaic, which not only prevented an increase in glycerides but also in cholesterol. The observations were confirmed by Longenecker, Gavin and McHenry (224) and later it was shown by Gavin and McHenry (92) that the same type of fatty liver could be produced by supplying biotin, in conjunction with thiamin, riboflavin, pantothenic acid and pyridoxine. The fatty liver thus produced was referred to as the biotin type. Subsequently, Gavin and McHenry (225) reported that the biotin fatty liver could be prevented by feeding inositol.

The demonstration that lipocaic would prevent the biotin fatty liver provided evidence, in addition to that furnished by Channon et al. (214), that lipotropic activity could be shown for lipocaic in rats, provided that a suitable type of fatty liver was used.

It has already been pointed out that there is some doubt that lipocaic should be considered to be a hormone. Additional evidence against such a classification was supplied by Gavin and McHenry (223). These workers made extracts from livers, muscle, wheat germ and rice polishings by the method described by Dragstedt et al. (207) for the preparation of crude lipocaic from pancreas; the extracts from all sources were active in preventing biotin fatty livers in rats. It should be made clear, however, that this is not definite evidence against Dragstedt's contention because Gavin and McHenry tested the extracts in rats with one type of fatty liver only; the extracts were not tried in depancreatized dogs. All of the extracts may have contained inositol and this substance may not be the constituent of pancreatic extracts which prevents fatty livers in dogs. The evidence for lipocaic being a hormone is not more satisfactory. While Dragstedt and associates fed raw brain and liver to depancreatized dogs no reference has been found to the preparation and use of even a partially purified extract from tissues other than pancreas. Admittedly the inclusion of raw brain or liver in the diet gave no evidence of lipotropic effect but these tissues may contain a constituent, e.g., biotin, which neutralizes the effect of any lipocaic, or inositol, which may be present also. The process used for the preparation of lipocaic might eliminate the lipogenic factor from the final extract, leaving only the lipotropic one. This would explain the results which Gavin and McHenry secured in rats and would cast considerable doubt upon the contention that lipocaic was a hormone. After reviewing the evidence it would appear that there is not satisfactory proof that lipocaic is an internal secretion of the pancreas.

The present situation with regard to the cause and prevention of fatty livers in depancreatized dogs is that there are a number of unanswered questions. Practically all of the information has been secured by four groups of investigators. It has previously been indicated that all fatty livers in rats are not alike in production, in composition, nor in response to lipotropic agents. Is there any reason for concluding that the cause and prevention of fatty livers in dogs maintained under different conditions are always identical? Best and associates (6) have emphasized the rôle of choline in dogs; it does not appear that they have tried lipocaic in depancreatized dogs. Their animals were maintained on a diet of raw lean beef

and sucrose, that is, a high-protein, high-carbohydrate, low-fat ration, and generally without additional vitamin supplements. Dragstedt et al. (217) have stressed the large dosage of choline which they found to be necessary and the satisfactory response given by lipocaic. Their animals were fed a ration of meat, whole milk and white bread and this contained appreciably more fat than that used by Best and associates. With dogs maintained on this diet Dragstedt has failed to find fatty livers after ligation of pancreatic ducts, or that pancreatic juice has a lipotropic effect. The basal rations employed by Chaikoff (218) and by Ralli (205) have differed from each other and from those supplied in other laboratories; both Chaikoff and Ralli have included generous amounts of the B vitamins in the diets. Great care should be exercised in comparing results between different species of animals but it might well be recalled that the inclusion of a minute amount of biotin in the diet fed to rats causes a marked difference in response to lipotropic agents. It is very difficult, if not impossible, to compare the results found in different laboratories in studies on depancreatized dogs in view of the wide variation in the basal rations. In the opinion of the present reviewers clarification will not be secured until standardization of technique is adopted, particularly with reference to the basal ration.

There are a number of attractive, unsolved questions in the investigations on depancreatized dogs. The work of Ralli and Rubin (222), and that by Gavin and McHenry (92) in rats, suggests that there may be a positive agent promoting fat deposition in the liver. Is this agent biotin? Biotin causes fatty livers in rats which resemble in several respects at least some of the fatty livers which have been studied in dogs. What is the rôle of pancreatic juice? One is inclined to accept the finding of Chaikoff and associates (218–221) and of Ralli et al. (215) that the absence of pancreatic juice contributes to the formation of fatty livers. Pancreatic juice may supply lipocaic directly or it may cause the liberation and consequent absorption of a lipotropic factor from foods in the basal diet. While the contention that lipocaic is a hormone may be doubted, there can be no question that it is lipotropic, nor that this activity is due to a constituent other than choline or protein. What is the chemical nature of lipocaic? Very little information is available regarding the active substance. The production and prevention of fatty livers in depancreatized dogs still offers fruitful field for investigation.

III. Inositol. In 1928 Eastcott (226), working in Lash Miller's laboratory, identified bios I as inactive inositol. Woolley (227) showed in 1940 that inositol is an essential dietary factor for mice and that a deficiency of it causes alopecia in that species. Deficiency symptoms have also been described in rats by Pavcek and Baum (228) who believed that a lack of inositol causes the so-called "spectacle-eye" syndrome.

Gavin and McHenry (225) reported in 1941 that the inclusion of inositol in the diets of white rats would prevent the biotin type of fatty liver, caused by the administration of biotin in conjunction with thiamin, riboflavin, pantothenic acid and pyridoxine. Inositol not only prevented the accumulation of fatty acids but also the increase in cholesterol content which is characteristic of this type of fatty

liver. It was stated that this lipotropic action of inositol resembled that of lipocaic but it was not said that lipocaic owed its activity to inositol contained in the preparation, although such an inference could have been drawn.

Attempts to demonstrate a lipotropic effect of inositol in depancreatized dogs were subsequently reported by two groups of workers (Owens, Allen, Stringer and Dragstedt, 229; Rubin and Ralli, 230). Owens et al. found that inositol had some lipotropic effect but not as much as was shown by an identical amount of lipocaic. Inositol appeared to have little influence upon prolongation of life, upon insulin tolerance, or upon body fat; lipocaic did affect these. It was inferred that, while lipocaic may contain inositol, the various effects produced by it in depancreatized dogs are not given by inositol and that lipocaic probably contains a quite different, unidentified, lipotropic substance. A somewhat similar conclusion was drawn by Rubin and Ralli. Because of the extensive experience of both groups of investigators one is inclined to accept these results, although it should be pointed out that a small number of dogs was used in both cases and that it was not possible to try a great range of dosage of inositol. If the results are accepted, the conclusion is valid that lipocaic contains an unidentified substance. It may be added that preparations of lipocaic examined in the reviewers' laboratory have contained sufficient inositol to account for their lipotropic potency in rats.

Engel (8) has studied the effects of inositol in preventing fatty livers in rats. The animals received thiamin, riboflavin, pyridoxine, pantothenic acid, corn oil and choline in various dosages. A maximal effect was obtained with 10 mgm. choline per day but this amount did not ensure a normal quantity of liver fat. This was secured by providing 3 mgm. inositol in addition to choline. The lipotropic action of inositol, demonstrated by Gavin and McHenry, was corroborated. Although Engel did not use biotin, his results show that a type of fatty liver refractory to choline was secured and that inositol was effective under the conditions used. A more recent paper by Gavin, Patterson and McHenry (93) describes several circumstances under which inositol is effective. Since this will be discussed in a subsequent section, detailed reference will not be made to it here.

Although there is little experimental evidence regarding the mechanism by which inositol exerts a lipotropic action, some interesting speculations may be made. Anderson and associates (231, 232) showed that inositol was present in phospholipid combination in the tubercle bacillus and in soy beans. Folch and Woolley (134) reported that inositol was contained in considerable amounts in naturally occurring phospholipids of animal tissues. It might be suggested that inositol, like choline, owes its lipotropic activity to participation in the formation of certain phospholipids. It is of interest that inositol is effective under conditions unfavorable for the action of choline, particularly when large amounts of cholesterol are present in the liver. Complete analyses of inositol- and choline-containing phospholipids might provide valuable information regarding the mode of action of the two factors. It might be possible, for example, that a different selection of fatty acids is made to form inositol phospholipids from those

which combine with choline. Inositol would appear to have some relation to cholesterol metabolism but the relationship is unknown.

COMPARISON OF THE EFFECTS OF LIPOTROPIC FACTORS. It seems advantageous to bring together available information regarding the types of fatty livers which are affected by the three principal lipotropic factors. For this purpose it is assumed that with choline may be grouped substances like methionine which enable choline to be synthesized *in vivo*, and also compounds which are related to choline, like triethyl choline. The information has been summarized in table 3. Separate literature references are not provided because the activity given in the table is the reviewers' opinion based on an examination of a large number of papers already listed. In some cases the activity has been verified in two or more laboratories; such instances are noted with an asterisk. In those cases in

TABLE 3

Comparison of the effects of lipotropic factors

REGIMEN USED FOR PRODUCTION OF FATTY LIVERS	CHOLINE	LIPOCAIC	INOSITOL
Depancreatized dogs	++*	++*	−?
Rats:			
High fat diet, thiamin	++*	0	−
High fat diet, all B vitamins	++*	−	−
High fat diet, cholesterol	+*	0	+
Fat-free diet, thiamin	++*	−	0
Fat-free diet, thiamin and riboflavin	++*	−	−
Fat-free diet, thiamin, riboflavin, pyridoxine and pantothenic acid	+*	−	+*
Fat-free diet, above 4 vitamins and biotin	0	++	++
Fat-free diet, B vitamins and cholesterol	+	+	+

Note: ++ Strong lipotropic action.
+ Moderate lipotropic action.
0 No lipotropic action.
− Lack of data.
* Verified in two or more laboratories.

which the activity has been described in one report only there may be some doubt as to the validity of the conclusion.

This comparative table has been prepared for several purposes. It summarizes existing information in a small space. It draws attention to the fact, which has often been ignored, that all fatty livers are not alike in their response to lipotropic agents. In doing this, questions may be raised as to the differences which probably exist in the composition of the fats present in the livers. It will be noted that in the small number of instances in which comparison has been made there is some correspondence between the effects of lipocaic and of inositol. The similarity is possibly not true in depancreatized dogs and there seems to be a distinct difference with regard to fatty livers produced by a high-fat diet containing cholesterol. The latter instance is based on one report from one laboratory and there is no confirmation.

Why should an alteration in the B vitamin supplements lessen the effect of choline and make it possible for inositol to exert a lipotropic action? Possibly this is due to a change in the fatty acid composition of the liver. One might speculate that choline will combine with certain fatty acids to form a phospholipid, while inositol selects quite different acids. Some information could be secured by analysis of the two types of phospholipids. There is practically no information regarding the mechanism by which lipocaic causes fat to be removed from the liver. It should be pointed out that lipocaic and inositol, unlike choline, have a definite effect upon cholesterol metabolism. Unfortunately, in the case of lipocaic, there is still no information regarding the chemistry of the active constituent.

SUMMARY

Under normal conditions the amount of fat in the liver is fairly constant. An accumulation of excess fat is evidence of an alteration in metabolism. In general it may be said that the production of a fatty liver is due to either a failure in transport of fat from the liver or to a rapid withdrawal of fat from body stores at a rate so great that the liver is unable to cope with the fat brought to it. Hershey, in his initial studies on fatty livers in depancreatized dogs, assumed that a failure in transport was responsible. Accepting the thesis that phospholipids were essential for fat transport, Hershey fed lecithin to his animals and secured a marked reduction in liver fat. These results gave rise to extensive investigations, in a number of laboratories, on the cause and prevention of fatty livers. Best and associates showed that it was unnecessary to feed lecithin since one constituent of the phospholipid, choline, was sufficient to restore conditions to normal. The obvious, and most simple, explanation of the lipotropic action of choline, that it promoted the formation of phospholipids, was validated by the work of Welch and of Perlman and Chaikoff. A molecular structure similar to that of choline in general configuration appears to be necessary for the formation of phospholipids of the lecithin type; various substitutions may be made within the molecule with retention of lipotropic activity. The effect of protein upon fat transport has been satisfactorily explained as being due to the supply of methyl groups from methionine for the synthesis of choline, for which process ethanolamine must also be available. The investigations on transmethylation have shown, as well, that choline may furnish methyl groups for several biological processes. This is the second function of choline for which there is definite evidence but it is one which is not related to lipotropic activity. A third, and obvious, rôle for choline has been suggested, the formation of acetylcholine. There have been described a number of effects of choline deficiency which are not clearly related to any of the three functions ascribed to choline. It would seem likely that some of these may be due to the failure of fat transport; this is particularly probable in the case of kidney lesions.

While it appeared likely for a short time that choline was the only constituent of pancreas which reduced the amount of liver fat in depancreatized dogs, the work of Dragstedt and associates has established the presence of another lipotropic substance in pancreas, a factor to which Dragstedt gave the name lipocaic.

The chemistry and mode of action of lipocaic are unknown. In contrast to most early reports, investigations in the reviewers' laboratory have shown that the lipotropic effect of lipocaic can be easily demonstrated in rats, provided that a suitable type of fatty liver is used. It should be recognized that all fatty livers are not alike and that the action of a lipotropic agent is conditioned by the character of the lipids in the liver. The contention that lipocaic is a hormone has not been substantiated. Further investigations, particularly of a biochemical nature, upon the mechanism of production of fatty livers in depancreatized animals and upon the function of lipocaic would be advantageous.

It has been established that inositol acts as a lipotropic factor in the rat and it seems likely that this activity is due, like that of choline, to the formation of phospholipids. An alteration in the B vitamin supplements will so change a fatty liver that it is resistant to the action of choline but susceptible to that of inositol. It would be interesting to determine the changes which are produced in the liver lipids, and which probably determine the activity of the lipotropic agents.

While the investigations on lipotropic factors have not, as yet, yielded results which can be applied to the treatment of disease in humans, they have opened a new approach to the study of fat metabolism. The chief value of the work on the lipotropic action of choline has been to provide additional proof for the part played by phospholipids in fat transport. New information has been made available regarding transmethylation, about the effects of B vitamins upon fat metabolism, and concerning other lipotropic factors. The investigations have shown the paucity of our knowledge of fat metabolism and have indicated questions for further research.

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